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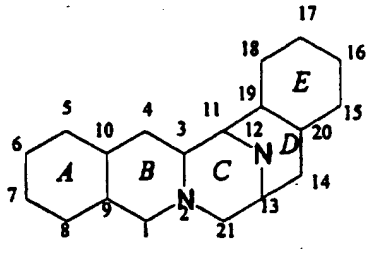
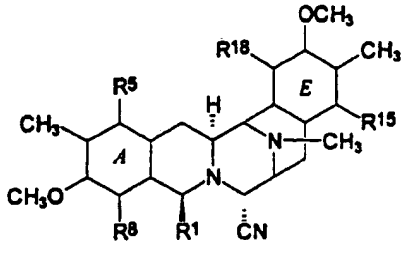
(54) Title: HEMISYNTHETIC METHOD AND NEW COMPOUNDS

(57) Abstract

Methods are provided for preparing a compound with a fused ring structure of formula (XIV) which comprises one or more reactions starting from a 21-cyano compound of formula (XVI) where typically: $R^1$ is an amidomethylene group or an acyloxymethylene group; $R^5$ and $R^8$ are independently chosen from -H, -OH or $-OCOCH_2OH$, or $R^5$ and $R^8$ are both keto and the ring *A* is a p-benzoquinone ring; $R^{14a}$ and $R^{14b}$ are both -H ozone is -H and the other is -OH, $-OCH_3$ or $-OCH_2CH_3$, or $R^{14a}$ and $R^{14b}$ together form a keto group; and $R^{15}$ and $R^{18}$ are independently chosen from -H or -OH, or $R^5$ and $R^8$ are both keto and the ring *A* is a p-benzoquinone ring. In modified starting materials, the 21-cyano group can be replaced by other groups introduced using nucleophilic reagents.




(XIV)




(XVI)

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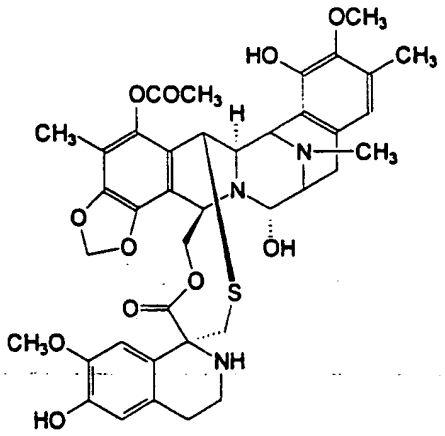
# HEMISYNTHETIC METHOD AND NEW COMPOUNDS

The present invention relates to synthetic methods, and in particular it relates to hemisynthetic methods.

## BACKGROUND OF THE INVENTION

European Patent 309,477 relates to ecteinascidins 729, 743, 745, 759A, 759B and 770. The ecteinascidin compounds are disclosed to have antibacterial and other useful properties. Ecteinascidin 743 is now undergoing clinical trials as an antitumour agent.

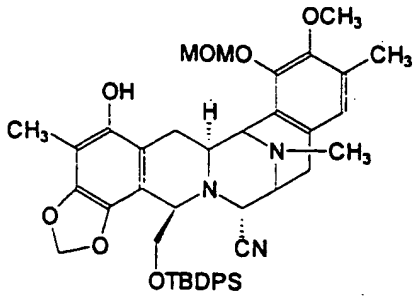
Ecteinascidin 743 has a complex tris(tetrahydroisoquinolinephenol) structure of the following formula (I):




It is currently prepared by isolation from extracts of the marine tunicate *Ecteinascidin turbinata*. The yield is low, and alternative preparative processes have been sought.

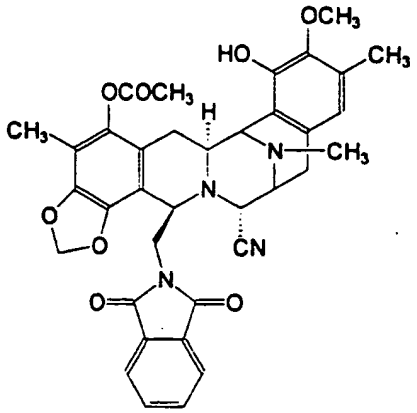
A synthetic process for producing ecteinascidin compounds is described in US Patent 5,721,362. The claimed method is long and complicated, there being 38 Examples each describing a step in the synthetic sequence to arrive at ecteinascidin 743.

Claim 25 of US 5,721,362 is directed at an intermediate phenol compound of a given formula (11), which we refer to also as Intermediate 11 or Int-11. It has the following bis(tetrahydroisoquinolinephenol) structure (II):

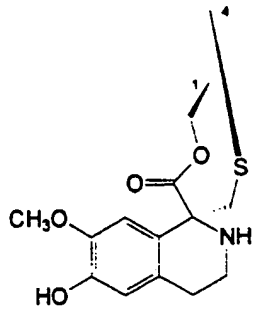



where MOM is a methoxymethyl substituent and TBDPS is a 3,5-t-butyldiphenylsilyl substituent.

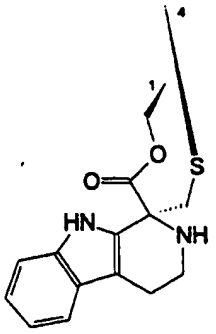
From Intermediate 11 it is possible to synthesise another interesting antitumour agent, phthalascidin, see Proc. Natl. Acad. Sci. USA, 96, 3496-3501, 1999. Phthalascidin is a bis(tetrahydroisoquinolinephenol) derivative of formula (III):



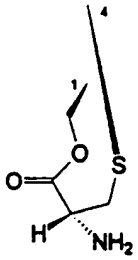

In ecteinascidin 743, the 1,4 bridge has the structure of formula (IV):



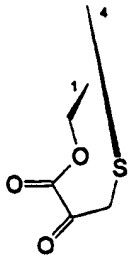

Other known ecteinascidins include compounds with a different bridged cyclic ring system, such as occurs in ecteinascidin 722 and 736, where the bridge has the structure of formula (V):




ecteinascidins 583 and 597, where the bridge has the structure of formula (VI):




and ecteinascidin 594 and 596, where the bridge has the structure of formula (VII):




The complete structure for these and related compounds is given in J. Am. Chem. Soc.

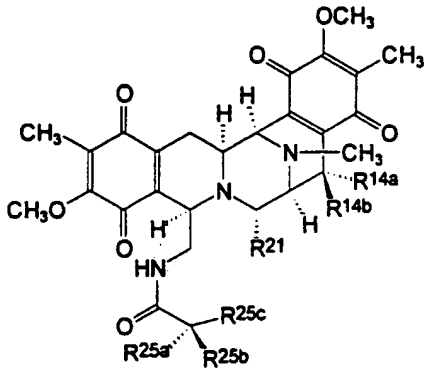
(1996) 118, 9017-9023. This article is incorporated by reference.

Further compounds are known which lack a bridged cyclic ring system. They include the bis(tetrahydroisoquinolinequinone) antitumor-antimicrobial antibiotics safracins and saframycins, and the marine natural products renieramicins and xestomycin isolated from cultured microbes or sponges. They all have a common dimeric tetrahydroisoquinoline carbon framework. These compounds can be classified into four types, types I to IV, with respect to the oxidation pattern of the aromatic rings.

Type I, dimeric isoquinolinequinones, is a system of formula (VIII) most commonly occurring in this class of compounds, see the following table I.

Table I

Structure of Type I Saframycin Antibiotics.

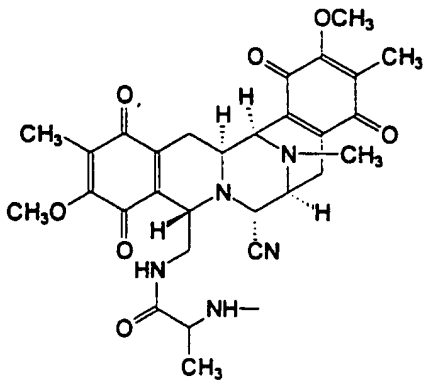



| | Substituents | | | | | |
|---|---|---|---|---|---|---|
| Compound | $R^{14a}$ | $R^{14b}$ | $R^{21}$ | $R^{25a}$ | $R^{25b}$ | $R^{25c}$ |
| saframycin A | H | H | CN | O | O | $CH_3$ |
| saframycin B | H | H | H | O | O | $CH_3$ |
| saframycin C | H | $OCH_3$ | H | O | O | $CH_3$ |
| saframycin G | H | OH | CN | O | O | $CH_3$ |
| saframycin H | H | H | CN | OH | $CH_2COCH_3$ | $CH_3$ |
| saframycin S | H | H | OH | O | O | $CH_3$ |
| saframycin $Y_3$ | H | H | CN | $NH_2$ | H | $CH_3$ |

| | | | | | | |
|---|---|---|---|---|---|---|
| saframycin $Yd_1$ | H | H | CN | $NH_2$ | H | $C_2H_5$ |
| saframycin $Ad_1$ | H | H | CN | O | O | $C_2H_5$ |
| saframycin $Yd_2$ | H | H | CN | $NH_2$ | H | H |
| saframycin $Y_{2b}$ | H | Q[b] | CN | $NH_2$ | H | $CH_3$ |
| saframycin $Y_{2b-d}$ | H | Q[b] | CN | $NH_2$ | H | $C_2H_5$ |
| saframycin $AH_2$ | H | H | CN | H[a] | OH[a] | $CH_3$ |
| saframycin $AH_2Ac$ | H | H | CN | H | OAc | $CH_3$ |
| saframycin $AH_1$ | H | H | CN | OH[a] | H[a] | $CH_3$ |
| saframycin $AH_1Ac$ | H | H | CN | OAc | H | $CH_3$ |
| saframycin $AR_3$ | H | H | H | H | OH | $CH_3$ |

[a] assignments are interchangeable.

[b] where the group Q is of formula (IX):



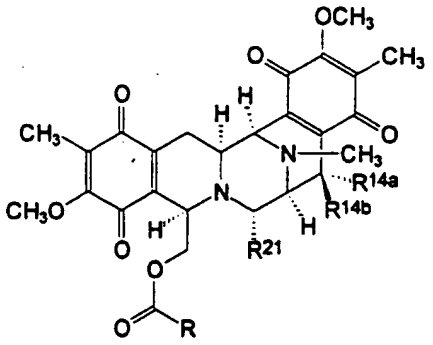

Type I aromatic rings are seen in saframycins A, B and C; G and H; and S isolated from *Streptomyces lavendulae* as minor components. A cyano derivative of saframycin A, called cyanoquinonamine, is known from Japanese Kokai JP-A2 59/225189 and 60/084288. Saframycins $Y_3$, $Yd_1$, $Ad_1$, and $Yd_2$ were produced by *S. lavendulae* by directed biosynthesis, with appropriate supplementation of the culture medium. Saframycins $Y_{2b}$ and $Y_{2b-d}$ dimers formed by linking the nitrogen on the C-25 of one unit to the C-14 of the other, have also been produced in supplemented culture media of *S. lavendulae*. Saframycins $AR_1$ (=$AH_2$,), a microbial reduction product of saframycin A at C-25 produced by *Rhodococcus amidophilus*, is also prepared by nonstereoselective chemical reduction of saframycin A by sodium borohydride as a 1:1 mixture of epimers followed by chromatographic separation [the other isomer $AH_1$ is less polar]. The further reduction product saframycin $AR_3$, 21-decyano-25-

dihydro-saframycin A, (= 25-dihydrosaframycin B) was produced by the same microbial conversion. Another type of microbial conversion of saframycin A using a *Nocardia* species produced saframycin B and further reduction by a *Mycobacterium* species produced saframycin $AH^1Ac$. The 25-*O*-acetates of saframycin $AH_2$ and $AH_1$ have also been prepared chemically for biological studies.

Type I compounds of formula (X) have also been isolated from marines sponges, see Table II.

Table II

Structures of Type I Compounds from Marine Sponges.




| | Substituents | | | |
|---|---|---|---|---|
| | $R^{14a}$ | $R^{14b}$ | $R^{21}$ | R |
| renieramycin A | OH | H | H | $-C(CH_3)=CH-CH_3$ |
| renieramycin B | $OC_2H_5$ | H | H | $-C(CH_3)=CH-CH_3$ |
| renieramycin C | OH | O | O | $-C(CH_3)=CH-CH_3$ |
| renieramycin D | $OC_2H_5$ | O | O | $-C(CH_3)=CH-CH_3$ |
| renieramycin E | H | H | OH | $-C(CH_3)=CH-CH_3$ |
| renieramycin F | $OCH_3$ | H | OH | $-C(CH_3)=CH-CH_3$ |
| xestomycin | $OCH_3$ | H | H | $-CH_3$ |

Renieramycins A-D were isolated from the antimicrobial extract of a sponge, a *Reniera* species collected in Mexico, along with the biogenetically related monomeric isoquinolines renierone and related compounds. The structure of renieramycin A was

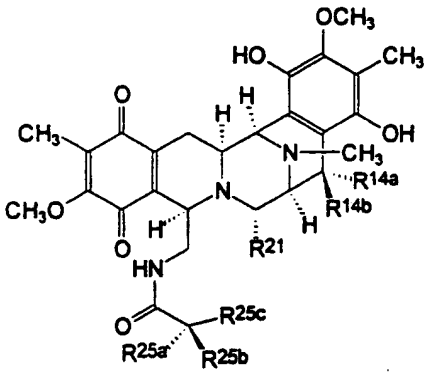
initially assigned with inverted stereochemistry at C-3, C-11, and C-13. However, careful examination of the $^1$H NMR data for new, related compounds renieramycins E and F, isolated from the same sponge collected in Palau, revealed that the ring junction of renieramycins was identical to that of saframycins. This result led to the conclusion that the formerly assigned stereochemistry of renieramycins A to D must be the same as that of saframycins.

Xestomycin was found in a sponge, a *Xestospongia* species collected from Sri Lancan waters.

Type II compounds of formula (XI) with a reduced hydroquinone ring include saframycins D and F, isolated from *S. lavendulae*, and saframycins Mx-1 and Mx-2, isolated from *Myxococcus xanthus*. See table III.

Table III

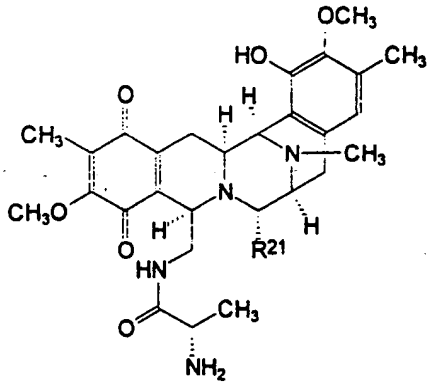
Type II Compounds




| | Substituents | | | | | |
|---|---|---|---|---|---|---|
| Compound | $R^{14a}$ | $R^{14b}$ | $R^{21}$ | $R^{25a}$ | $R^{25b}$ | $R^{25c}$ |
| saframycin D | O | O | H | O | O | $CH_3$ |
| saframycin F | O | O | CN | O | O | $CH_3$ |
| saframycin Mx-1 | H | $OCH_3$ | OH | H | $CH_3$ | $NH_2$ |
| saframycin Mx-2 | H | $OCH_3$ | H | H | $CH_3$ | $NH_2$ |

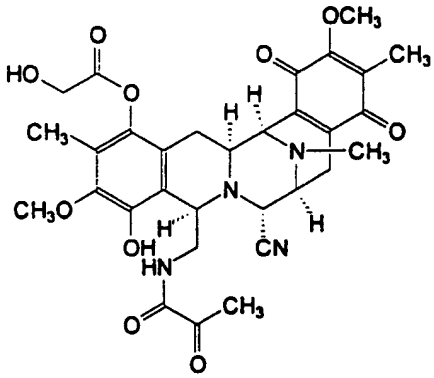
The type III skeleton is found in the antibiotics safracins A and B, isolated from

cultured *Pseudomonas fluorescens*. These antibiotics of formula (XII) consist of a tetrahydroisoquinoline-quinone subunit and a tetrahydroisoquninolinephenol subunit.

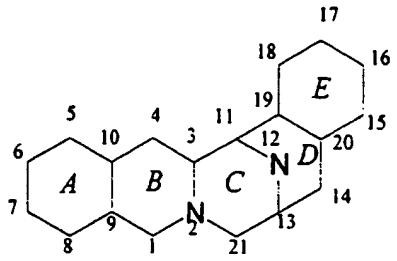



where $R^{21}$ is -H in safracin A and is -OH in safracin B.

Saframycin R, the only compound classified as the Type IV skeleton, was also isolated from *S. lavendulae*. This compound of formula (XIII), consisting of a hydroquinone ring with a glycolic ester sidechain on one of the phenolic oxygens, is conceivably a pro-drug of saframycin A because of its moderate toxicity.




All these known compounds have a fused system of five rings (*A*) to (*E*) as shown in the following structure of formula (XIV):




The rings *A* and *E* are phenolic in the ecteinascidins and some other compounds, while in other compounds, notably the saframycins, the rings *A* and *E* are quinolic. In the known compounds, the rings *B* and *D* are tetrahydro, while ring *C* is perhydro.

## OBJECT OF THE INVENTION

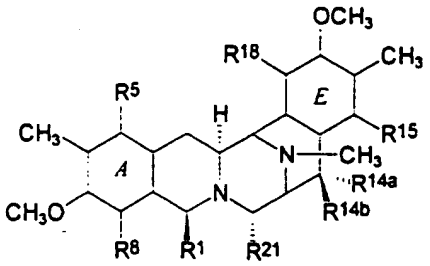
The need remains for new active compounds with the fused five-ring system of the known compounds, and for alternative synthetic routes to the ecteinascidin compounds and related compounds. Such synthetic routes may provide more economic paths to the known antitumour agents, as well as permitting preparation of new active compounds.

## SUMMARY OF THE INVENTION

In one aspect, the present invention is directed at the use of a known compound, safracin B, also referred to as quinonamine, in hemisynthetic synthesis.

More generally, the invention relates to a hemisynthetic process for the formation of intermediates, derivatives and related structures of ecteinascidin or other tetrahydroisoquinolinephenol compounds starting from natural bis(tetrahydroisoquinoline) alkaloids. Suitable starting materials for the hemi-synthetic process include the classes of saframycin and safracin antibiotics available from different culture broths, and also the classes of reineramicin and xestomycin compounds available from marine sponges.

A general formula (XV) for the starting compounds is as follows:




where:

$R^1$ is an amidomethylene group such as $-CH_2-NH-CO-CR^{25a}R^{25b}R^{25c}$ where $R^{25a}$ and $R^{25b}$ form a keto group or one is -OH, $-NH_2$ or $-OCOCH_3$ and the other is $-CH_2COCH_3$, -H, -OH or $-OCOCH_3$, provided that when $R^{25a}$ is -OH or $-NH_2$ then $R^{25b}$ is not -OH, and $R^{25c}$ is -H, $-CH_3$ or $-CH_2CH_3$, or $R^1$ is an acyloxymethylene group such as $-CH_2-O-CO-R$, where R is $-C(CH_3)=CH-CH_3$ or $-CH_3$;

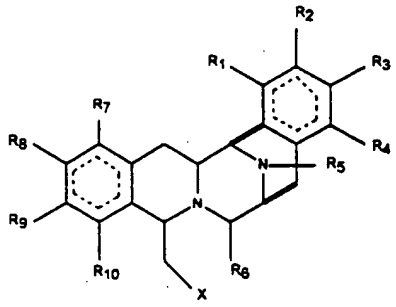
$R^5$ and $R^8$ are independently chosen from -H, -OH or $-OCOCH_2OH$, or $R^5$ and $R^8$ are both keto and the ring *A* is a p-benzoquinone ring;

$R^{14a}$ and $R^{14b}$ are both -H or one is -H and the other is -OH, $-OCH_3$ or $-OCH_2CH_3$, or $R^{14a}$ and $R^{14b}$ together form a keto group;

$R^{15}$ and $R^{18}$ are independently chosen from -H or -OH, or $R^5$ and $R^8$ are both keto and the ring *A* is a p-benzoquinone ring; and

$R^{21}$ is -OH or -CN.

A more general formula for these class of compounds is provided below:



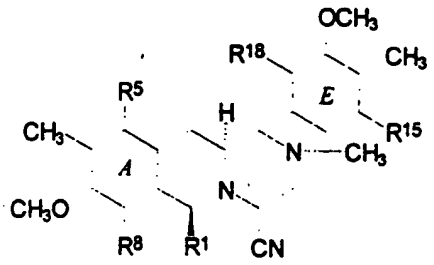

wherein the substituent groups defined by $R_1$, $R_2$, $R_3$, $R_4$, $R_5$, $R_6$, $R_7$, $R_8$, $R_9$, $R_{10}$ are each independently selected from the group consisting of H, OH, $OCH_3$, CN, =O, $CH_3$;

wherein X are the different amide or ester functionalities contained in the mentioned natural products;

wherein each dotted circle represents one, two or three optional double bonds.

Thus, according to the present invention, we now provide hemisynthetic routes for the production of intermediates including Intermediate 11 and thus for the production of the ecteinascidin compounds as well as phthalascidin and additional compounds. The hemisynthetic routes of the invention each comprise a number of transformation steps to arrive at the desired product. Each step in itself is a process in accordance with this invention. The invention is not limited to the routes that are exemplified, and alternative routes may be provided by, for example, changing the order of the transformation steps, as appropriate.

In particular, this invention involves the provision of a 21-cyano starting material of general formula (XVI):




where $R^1$, $R^5$, $R^8$, $R^{14a}$, $R^{14b}$, $R^{15}$ and $R^{18}$ are as defined.

Other compounds of formula (XVI) with different substituents at the 21-position may also represent possible starting materials. In general, any derivative capable of production by nucleophilic displacement of the 21-hydroxy group of compounds of formula (XV) wherein $R^{21}$ is a hydroxy group cis a candidate. Examples of suitable 21-substituents include but are not limited to:

a mercapto group;

an alkylthio group (the alkyl group having from 1 to 6 carbon atoms);

an arylthio group (the aryl group having from 6 to 10 carbon atoms and being unsubstituted or substituted by from 1 to 5 substituents selected from, for example, alkyl group having from 1 to 6 carbon atoms, alkoxy groups having from 1 to 6 carbon atoms, halogen atoms, mercapto groups and nitro groups);

an amino group;

a mono-or dialkylamino (the or each alkyl group having from 1 to 6 carbon atoms);

a mono-or diarylamino group (the or each aryl group being as defined above in relation to arylthio groups);

an α-carbonylalkyl group of formula $-C(R^a)(R^b)-C(=O)R^c$, where

$R^a$ and $R^b$ are selected from hydrogen atoms, alkyl groups having from 1 to 20 carbon atoms, aryl groups (as defined above in relation to arylthio groups) and aralkyl groups (in which an alkyl group having from 1 to 4 carbon atoms is substituted by an aryl group a defined above in relation to arylthio groups), with the proviso that one of $R^a$ and $R^b$ is a hydrogen atom;

$R^c$ is selected from a hydrogen atom, an alkyl group having from 1 to 20 carbon atoms, aryl groups (as defined above in relation to arylthio groups), an aralkyl group (in which an alkyl group having from 1 to 4 carbon atoms is substituted by an aryl group a defined above in relation to arylthio groups), an alkoxy group having from 1 to 6 carbon atoms, an amino group or a mono- or dialkylamino group as defined above.

Thus, in a more general aspect, the present invention relates to processes where the first step is to form a 21-deriviative using a nucleophilic reagent. We refer to such compounds as 21-Nuc compounds.

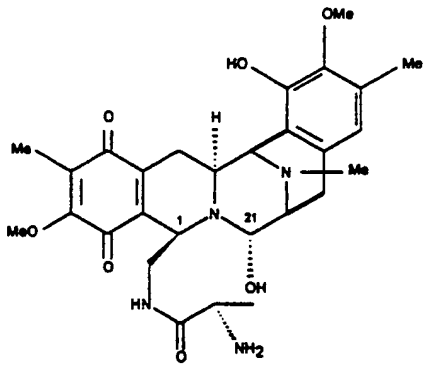
The presence of the 21-cyano group is required for some of the end-products, notably ecteinascidin 770 and phthalascidin, while for other end-products it acts as a protecting group which can readily be converted to another substituent, such as the 21-hydroxy group of ecteinascidin 743 or of 21-hydroxyphthalascidin. The adoption of the 21-cyano compound as the starting material effectively stabilises the molecule during the ensuing synthetic steps, until it is optionally removed. Other 21-Nuc compounds can offer this and other advantages.

In one important aspect, the present invention consists in the use of a 21-cyano compound of the general formula (XVI) in the preparation of a bis- or tris-(tetrahydroisoquinolinephenol) compounds. Products which may be prepared include intermediates such as Intermediate 11, and the ecteinascidins and phthalascidin, as well as new and known compounds of related structure.

Preferred starting materials include those compounds of formula (XV) or (XVI) where $R^{14a}$ and $R^{14b}$ are both hydrogen. Preferred starting materials also include compounds of formula (XV) or (XVI) where $R^{15}$ is hydrogen. Furthermore, the preferred starting materials include compounds of formula (XV) or (XVI) where ring *E* is a phenolic ring. Preferred starting materials further include compounds of formula (XV) or (XVI) where at least one, better at least two or three of $R^5$, $R^8$, $R^{15}$ and $R^{18}$ is not hydrogen.

Examples of suitable starting materials for this invention include saframycin A, saframycin B, saframycin C, saframycin G, saframycin H, saframycin S, saframycin $Y_3$, saframycin $Yd_1$, saframycin $Ad_1$, saframycin $Yd_2$, saframycin $AH_2$, saframycin $AH_2Ac$, saframycin $AH_1$, saframycin $AH_1Ac$, saframycin $AR_3$, renieramycin A, renieramycin B, renieramycin C, renieramycin D, renieramycin E, renieramycin F, xestomycin, saframycin D, saframycin F, saframycin Mx-1, saframycin Mx-2, safracin A, safracin B and saframycin R. Preferred starting materials have a cyano group in position 21, for the group $R^{21}$.

In a particularly preferred aspect, the invention involves a hemisynthetic process wherein the transformation steps are applied to safracin B:



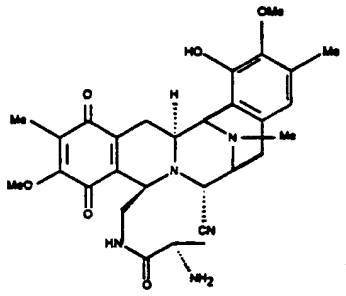

SAFRACIN B

Safracin B presents a ring system closely related to the ecteinascidins. This compound has the same pentacycle structure and the same substitution pattern in the right-hand aromatic ring, ring *E*. Also, safracin B presents very close similarities to some of the synthetic intermediates in the total synthesis of ET-743, particularly to the intermediate 11.

Such intermediate can be transformed into Et-743 using a well established method. Synthetic conversion of safracin B into intermediate 11 will therefore provide an hemi-synthetic method to obtain ET-743.

Thus, we provide Intermediate 11 made from this compound safracin B, and compounds derived from Intermediate 11, particularly ecteinascidin compounds. We further provide phthalascidin made from safracin B. The invention also relates to use of safracin B in the production of Intermediate 11, phthalascidin, ecteinascidin compounds and the other intermediates of the invention. The invention also relates to compounds described herein derived from the other suggested starting materials, and use of those compounds in the production of such compounds.

The more preferred starting materials of this invention have a 21-cyano group. The currently most preferred compound of the present invention is the compound of Formula **2**. This compound is obtained directly from safracin B and is considered a key intermediate in the hemisynthetic process.

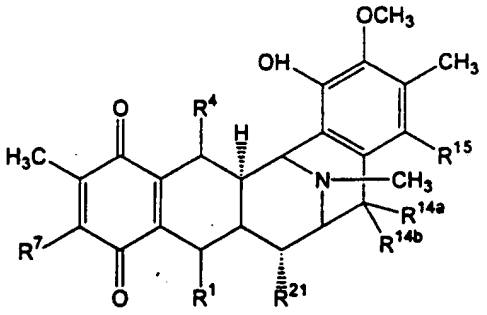


compound 2

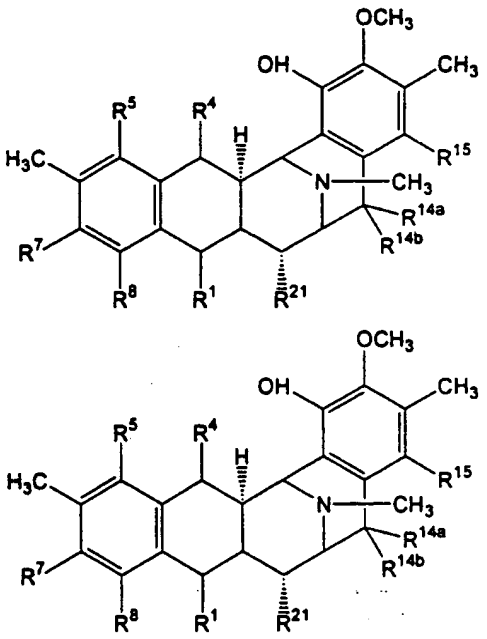
In a related aspect, we provide cyanosafracin B by fermentation of a safracin B-producing strain of *Pseudomonas fluorescens*, and working up the cultured broth using cyanide ion. The preferred strain of *Pseudomonas fluorescens* is strain A2-2, FERM BP-14, which is employed in the procedure of EP 055,299. A suitable source of cyanide ion is potassium cyanide. In a typical work-up, the broth is filtered and excess cyanide ion is added. After an appropriate interval of agitation, such as 1 hour, the pH is rendered alkaline, say pH 9.5, and an organic extraction gives a crude extract which can be further purified to give the cyanosafracin B.

For the avoidance of doubt, the stereochemistries indicated in this patent specification are based on our understanding of the correct stereochemistry of the natural products. To the extent that an error is discovered in the assigned stereochemistry, then the appropriate correction needs to be made in the formulae given throughout in this patent specification. Furthermore, to the extent that the syntheses are capable of modification, this invention extends to stereoisomers.

The products of this invention are typically of the formula (XVIIa):



or formula (XVIIb):



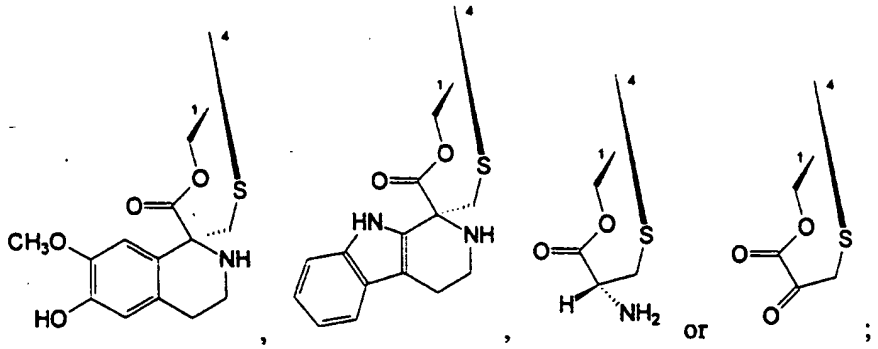
where

$R^1$ is an optionally protected or derivatised aminomethylene group, an optionally protected or derivatised hydroxymethylene group, such as a group $R^1$ as defined for the formula (XV);

$R^4$ is -H;

or

$R^1$ and $R^4$ together form a group of formula (IV), (V) (VI) or (VII):



$R^5$ is -H or -OH;

$R^7$ is $-OCH_3$ and $R^8$ is -OH or $R^7$ and $R^8$ together form a group $-O-CH_2-O-$;

$R^{14a}$ and $R^{14b}$ are both -H or one is -H and the other is -OH, $-OCH_3$ or $-OCH_2CH_3$, or $R^{14a}$ and $R^{14b}$ together form a keto group; and

$R^{15}$ is -H or -OH;

$R^{21}$ is -H, -OH or -CN;

and derivatives including acyl derivatives thereof especially where $R^5$ is acetyloxy or other acyloxy group of up to 4 carbon atoms, and including derivatives where the group $-NCH_3-$ at the 12-position is replaced by -NH- or $-NCH_2CH_3-$, and derivatives where the $-NH_2$ group in the compound of formula (VI) is optionally derivatised.

In the formulae (XVIIa) or (XVIIb), $R^1$ is typically aminomethylene, amidomethylene or $R^1$ with $R^4$ forms a group (IV) or (V). Suitable amidomethylene groups include those of formula $-CH_2-NH-CO-CHCH_3-NH_2$ derived from alanine, and similar groups derived from other amino acids, notably , both D and L, glycine, valine, leucine, isoleucine, phenylalanine, tyrosine, tryptophan, methionine, cysteine, aspartate, asparagine, glutamatic acid, glutamine, lysine, arginine, proline, serine, threonine, histidine and hydroxyproline. A general formula for the group $R^1$ is then $-CH_2-NH$ -aa, where aa indicates an acyl amino acid group.

The group $R^1$ can be acylated on an $-NH_2$ group, and for example N-acyl derivatives can be formed from groups $-CH_2NH_2$ and $-CH_2-NH$-aa. The acyl derivatives can be N-acyl or N-thioacyl derivatives thereof, as well as cyclic amides. The acyl groups can illustratively

be alkanoyl, haloalkanoyl, arylalkanoyl, alkenoyl, heterocyclylacyl, aroyl, arylaroyl, haloaroyl, nitroaroyl, or other acyl groups. The acyl groups can be of formula -CO-$R^a$, where $R^a$ can be various groups such as alkyl, alkoxy, alkylene, arylalkyl, arylalkylene, amino acid acyl, or heterocyclyl, each optionally substituted with halo, cyano, nitro, carboxyalkyl, alkoxy, aryl, aryloxy, heterocyclyl, heterocyclyloxy, alkyl, amino or substituted amino. Other acylating agents include isothiocyanates, such as aryl isothiocyanates, notably phenyl isocyanate. The alkyl, alkoxy or alkylene groups of $R^a$ suitably have 1 to 6 or 12 carbon atoms, and can be linear, branched or cyclic. Aryl groups are typically phenyl, biphenyl or naphthyl. Heterocyclyl groups can be aromatic or partially or completely unsaturated and suitably have 4 to 8 ring atoms, more preferably 5 or 6 ring atoms, with one or more heteroatoms selected from nitrogen, sulphur and oxygen.

Without being exhaustive, typical $R^a$ groups include alkyl, haloalkyl, alkoxyalkyl, haloalkoxyalkyl, arylalkylene, haloalkylarylakylene, acyl, haloacyl, arlyalkyl, alkenyl and amino acid. For example, $R^a$-CO- can be acetyl, trifluoroacetyl, 2,2,2-trichloroethoxycarbonyl, isovalerylcarbonyl, trans-3-(trifluoromethyl)cinnamoylcarbonyl, heptafluorobutyrylcarbonyl, decanoylcarbonyl, trans-cinnamoylcarbonyl, butyrylcarbonyl, 3-chloropropyonylcarbonyl, cinnamoylcarbonyl, 4-methylcinnamoylcarbonyl, hydrocinnamoylcarbonyl, or trans-hexenoylcarbonyl, or alanyl, arginyl, aspartyl, asparagyl, cystyl, glutamyl, glutaminyl, glycyl, histidyl, hydroxyprolyl., isoleucyl, leucyl, lysyl, methionyl, phenylalanyl, prolyl, seryl, threonyl, thyronyl, tryptophyl, tyrosyl, valyl, as well as other less common amino acid acyl groups, as well as phthalimido and other cyclic amides. Other examples may be found among the listed protecting groups.

Compounds wherein -CO-$R^a$ is derived from an amino acid and include an amino group can themselves form acyl derivatives. Suitable N-acyl commands include dipeptides which in turn can form N-acyl derivatives.

In one variation which relates to intermediate products, the ring A is modified to incorporate the substructure shown as formula (XX) or (XXI), discussed later.

In another variation relating to intermediates, the group $R^1$ can be

$-CH_2O-CO-CFu-CH_2-S-Prot^3$, derived from a compound of formula (XIX), where $Prot^3$ and Fu have the indicated meanings. In such a case, $R^7$ and $R^8$ from the oxymethyleneoxy group. The group $R^{18}$ is usually protected. Usually $R^{21}$ is cyano.

Preferably $R^{14a}$ and $R^{14b}$ are hydrogen. Preferably $R^{15}$ is hydrogen. The O-acyl derivatives are suitably aliphatic O-acyl derivatives, especially acyl derivatives of 1 to 4 carbon atoms, and typically an O-acetyl group, notably at the 5-position.

Suitable protecting groups for phenols and hydroxy groups include ethers and esters, such as alkyl, alkoxyalkyl, aryloxyalkyl, alkoxyalkoxyalkyl, alkylsilylalkoxyalkyl, alkylthioalkyl, arylthioalkyl, azidoalkyl, cyanoalkyl, chloroalkyl, heterocyclic, arylacyl, haloarylacyl, cycloalkylalkyl, alkenyl, cycloalkyl, alyklarylalkyl, alkoxyarylalkyl, nitroarylalkyl, haloarylalkyl, alkylaminocarbonylarylalkyl, alkylsulfinylarylalky, alkylsilyl and other ethers, and arylacyl, aryl alkyl carbonate, aliphatic carbonate, alkylsulfinylarlyalkyl carbonate, alkyl carbonate, aryl haloalkyl carbonate, aryl alkenyl carbonate, aryl carbamate, alkyl phosphinyl, alkylphosphinothioyl, aryl phosphinothioyl, aryl alkyl sulphonate and other esters. Such groups may optionally be substituted with the previously mentioned groups in $R^1$.

Suitable protecting groups for amines include carbamates, amides, and other protecting groups, such as alkyl, arylalkyl, sulpho- or halo- arylalkyl, haloalkyl, alkylsilylalkyl, arylalkyl, cycloalkylalkyl, alkylarylalkyl, heterocyclylalkyl, nitroarylalkyl, acylaminoalkyl, nitroaryldithioarylalkyl, dicycloalkylcarboxamidoalkyl, cycloalkyl, alkenyl, arylalkenyl, nitroarylalkenyl, heterocyclylalkenyl, heterocyclyl, hydroxyheterocyclyl, alkyldithio, alkoxy- or halo- or alkylsulphinyl arylalkyl, hetercyclylacyl, and other carbamates, and alkanoyl, haloalkanoyl, arylalkanoyl, alkenoyl, heterocyclylacyl, aroyl, arylaroyl, haloaroyl, nitroaroyl, and other amides, as well as alkyl, alkenyl, alkylsilylalkoxyalkyl, alkoxyalkyl, cyanoalkyl, heterocyclyl, alkoxyarylalkyl, cycloalkyl, nitroaryl, arylalkyl, alkoxy- or hydroxy- arylalkyl, and many other groups. Such groups may optionally be substituted with the previously mentioned groups in $R^1$.

Examples of such protecting groups are given in the following tables.

protection for -OH group

| ethers | abbreviation |
|---|---|
| methyl | |
| methoxymethyl | MOM |
| benzyloxymethyl | BOM |
| methoxyethoxymethyl | MEM |
| 2-(trimethylsilyl)ethoxymethyl | SEM |
| methylthiomethyl | MTM |
| phenylthiomethyl | PTM |
| azidomethyl | |
| cyanomethyl | |
| 2,2-dichloro-1,1-difluoroethyl | |
| 2-chloroethyl | |
| 2-bromoethyl | |
| tetrahydropyranyl | THP |
| 1-ethoxyethyl | EE |
| phenacyl | |
| 4-bromophenacyl | |
| cyclopropylmethyl | |
| allyl | |
| propargyl | |
| isopropyl | |
| cyclohexyl | |
| *t*-butyl | |
| benzyl | |
| 2,6-dimethylbenzyl | |
| 4-methoxybenzyl | MPM or PMB |
| *o*-nitrobenzyl | |
| 2,6-dichlorobenzyl | |
| 3,4-dichlorobenzyl | |
| 4-(dimethylamino)carbonylbenzyl | |
| 4-methylsuflinylbenzyl | Msib |
| 9-anthrylmethyl | |
| 4-picolyl | |
| heptafluoro-*p*-tolyl | |
| tetrafluoro-4-pyridyl | |
| | |
| trimethylsilyl | TMS |
| *t*-butyldimethylsilyl | TBDMS |
| *t*-butyldiphenylsilyl | TBDPS |
| triisopropylsilyl | TIPS |

esters

| | |
|---|---|
| aryl formate | |
| aryl acetate | |
| aryl levulinate | |
| aryl pivaloate | ArOPv |
| aryl benzoate | |
| aryl 9-fluorocarboxylate | |
| | |
| aryl methyl carbonate | |
| 1-adamantyl carbonate | |
| *t*-butyl carbonate | BOC-OAr |
| 4-methylsulfinylbenzyl carbonate | Msz-Oar |
| 2,4-dimethylpent-3-yl carbonate | Doc-Oar |
| aryl 2,2,2-trichloroethyl carbonate | |
| aryl vinyl carbonate | |
| aryl benzyl carbonate | |
| aryl carbamate | |
| | |
| dimethylphosphinyl | Dmp-OAr |
| dimethylphosphinothioyl | Mpt-OAr |
| diphenylphosphinothioyl | Dpt-Oar |
| | |
| aryl methanesulfonate | |
| aryl toluenesulfonate | |
| aryl 2-formylbenzenesulfonate | |

protection for the -$NH_2$ group

| carbamates | abbreviation |
|---|---|
| | |
| methyl | |
| ethyl | |
| 9-fluorenylmethyl | Fmoc |
| 9-(2-sulfo)fluroenylmethyl | |
| 9-(2,7-dibromo)fluorenylmethyl | |
| 17-tetrabenzo[*a,c,g,i*]fluorenylmethyl | Tbfmoc |
| 2-chloro-3-indenylmethyl | Climoc |
| benz[*f*]inden-3-ylmethyl | Bimoc |
| 2,7-di-*t*-butyl[9-(10,10-dioxo-10,10,10,10-tetrahydrothioxanthyl)]methyl | DBD-Tmoc |
| 2,2,2-trichloroethyl | Troc |
| 2-trimethylsilylethyl | Teoc |
| 2-phenylethyl | hZ |
| 1-(1-adamantyl)-1-methylethyl | Adpoc |
| 2-chlooethyl | |
| 1,1-dimethyl-2-chloroethyl | |
| 1,1-dimethyl-2-bromoethyl | |

| | |
|---|---|
| 1,1-dimethyl-2,2-dibromoethyl | DB-*t*-BOC |
| 1,1-dimethyl-2,2,2-trichloroethyl | TCBOC |
| 1-methyl-1-(4-biphenyl)ethyl | Bpoc |
| 1-(3,5-di-*t*-butylphenyl)-1-1-methylethyl | *t*-Burmeoc |
| 2-(2'-and 4'-pyridyl)ethyl | Pyoc |
| 2,2-bis(4'-nitrophenyl)ethyl | Bnpeoc |
| *n*-(2-pivaloylamino)-1,1-dimethylethyl | |
| 2-[(2-nitrophenyl)dithio]-1-phenylethyl | NpSSPeoc |
| 2-(*n,n*-dicyclohexylcarboxamido)ethyl | |
| *t*-butyl | BOC |
| 1-adamantyl | 1-Adoc |
| 2-adamantyl | 2-Adoc |
| vinyl | Voc |
| allyl | Aloc or Alloc |
| 1-isopropylallyl | Ipaoc |
| cinnamyl | Coc |
| 4-nitrocinnamyl | Noc |
| 3-(3'-pyridyl)prop-2-enyl | Paloc |
| 8-quinolyl | |
| *n*-hydroxypiperidinyl | |
| alkyldithio | |
| benzyl | Cbz or Z |
| *p*-methoxybenzyl | Moz |
| *p*-nitrobenzyl | PNZ |
| *p*-bromobenzyl | |
| *p*-chlorobenzyl | |
| 2,4-dichlorobenzyl | |
| 4-methylsulfinylbenzyl | Msz |
| 9-anthrylmethyl | |
| diphenylmethyl | |
| phenothiazinyl-(10)-carbonyl | |
| *n'-p*-toluenesulfonylaminocarbonyl | |
| *n'*-phenylaminothiocarbonyl | |

amides

| | |
|---|---|
| formamide | |
| acetamide | |
| chloroacetamide | |
| trifluoroacetamide | TFA |
| phenylacetamide | |
| 3-phenylpropanamide | |
| pent-4-enamide | |
| picolinamide | |
| 3-pyridylcarboxamide | |
| benzamide | |
| *p*-phenylbenzamide | |
| *n*-phthalimide | |

| | |
|---|---|
| *n*-tetrachlorophthalimide | TCP |
| 4-nitro-*n*-phthalimide | |
| *n*-dithiasuccinimide | Dts |
| *n*-2,3-diphenylmaleimide | |
| *n*-2,5-dimethylpyrrole | |
| *n*-2,5-bis(triisopropylsiloxyl)pyrrole | BIPSOP |
| *n*-1,1,4,4-tetramethyldisiliazacyclopentante adduct | STABASE |
| 1,1,3,3-tetramethyl-1,3-disilaisoindoline | BSB |

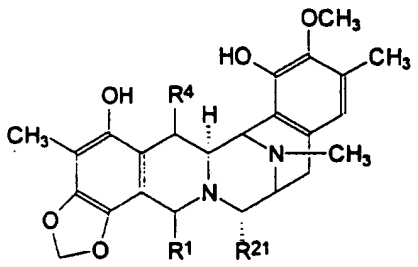
special -NH protective groups

| | |
|---|---|
| *n*-methylamine | |
| *n*-*t*-butylamine | |
| *n*-allylamine | |
| *n*-[2-trimethylsilyl)ethoxy]methylamine | SEM |
| *n*-3-acetoxypropylamine | |
| *n*-cyanomethylamine | |
| *n*-(1-isopropyl-4-nitro-2-oxo-3-pyrrolin-3-yl)amine | |
| *n*-2,4-dimethoxybenzylamine | Dmb |
| 2-azanorbornenes | |
| *n*-2,4-dinitrophenylamine | |
| *n*-benzylamine | Bn |
| *n*-4-methoxybenzylamine | MPM |
| *n*-2,4-dimethoxybenzylamine | DMPM |
| *n*-2-hydroxybenzylamine | Hbn |
| *n*-(diphenylmethyl)amino | DPM |
| *n*-bis(4-methoxyphenyl)methylamine | |
| *n*-5-dibenzosuberylamine | DBS |
| *n*-triphenylmethylamine | Tr |
| *n*-[(4-methoxyphenyl)diphenylmethyl]amino | MMTr |
| *n*-9-phenylflurenylamine | Pf |
| *n*-ferrocenylmethylamine | Fcm |
| *n*-2-picolylamine *n'*-oxide | |
| *n*-1,1-dimethylthiomethyleneamine | |
| *n*-benzylideneamine | |
| *n*-*p*-methoxybenzylideneamine | |
| *n*-diphenylmethyleneamine | |
| *n*-(5,5-dimethyl-3-oxo-1-cyclohexenyl)amine | |
| *n*-nitroamine | |
| *n*-nitrosoamine | |
| diphenylphosphinamide | Dpp |
| dimethylthiophosphinamide | Mpt |
| diphenylthiophosphinamide | Ppt |
| dibenzyl phosphoramidate | |
| 2-nitrobenzenesulfenamide | Nps |
| *n*-1-(2,2,2-trifluoro-1,1-diphenyl)ethylsufenamide | TDE |
| 3-nitro-2-pyridinesulfenamide | Npys |
| *p*-toluenesulfonamide | Ts |

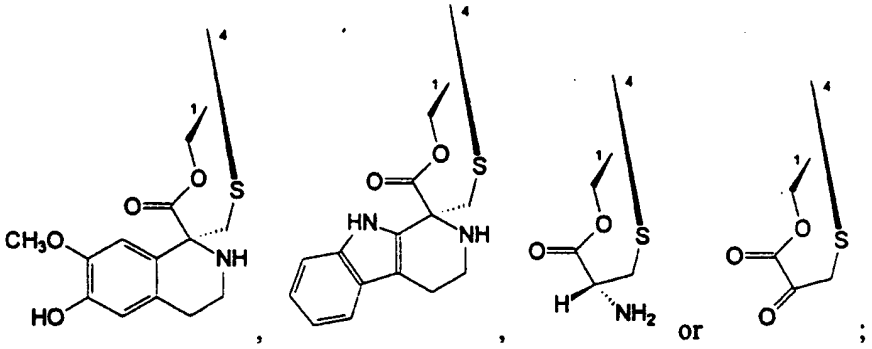
benzenesulfonamide

Safracin B includes an alanyl sidechain. In one aspect of the invention, we have found that protection of the free amino group with a Boc group can give strong advantages.

Particular ecteinascidin products of this invention include compounds of the formula (XVIII):



where $R^1$ and $R^4$ form a group of formula (IV), (V), (VI) or (VII):



more particularly a group (IV) or (V);

$R^{21}$ is -H, -OH or -CN, more particularly -OH or -CN;

and acyl derivatives thereof, more particularly 5-acyl derivatives including the 5-acetyl derivative.

FORMATION OF ECTEINASCIDIN 743 AND RELATED COMPOUNDS.

In general, the conversion of the 21-cyano starting compound to an ecteinascidin product of, for example, formula (XVIII) involves:

a) conversion if necessary of a quinone system for the ring *E* into the phenol system

b) conversion if necessary of a quinone system for the ring *A* into the phenol system;

c) conversion of the phenol system for the ring *A* into the methylenedioxyphenol ring;

d) formation of the bridged spiro ring system of formula (IV), (VI) or (VII) across the 1-position and 4-position in ring *B*; and

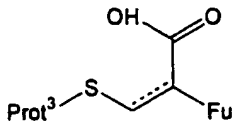
e) derivatisation as appropriate, such as acylation.

Step (a), conversion if necessary of a quinone system for the ring *E* into the phenol system, can be effected by conventional reduction procedures. A suitable reagent system is hydrogen with a palladium-carbon catalyst, though other reducing systems can be employed.

Step (b), conversion if necessary of a quinone system for the ring *A* into the phenol system is analogous to step (a), and more detail is not needed.

Step (c), conversion of the phenol system for the ring *A* into the methylenedioxyphenol ring, can be effected in several ways, possibly along with step (b). For example, a quinone ring A can be demethylated in the methoxy substituent at the 7-position and reduced to a dihydroquinone and trapped with a suitable electrophilic reagent such as $CH_2Br_2$, $BrCH_2Cl$, or a similar divalent reagent directly yielding the methylenedioxy ring system, or with a divalent reagent such as thiocarbonyldiimidazol which yields a substituted methylenedioxy ring system which can be converted to the desired ring.

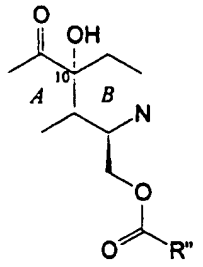
Step (d) is typically effected by appropriate substitution at the 1-position with a bridging reagent that can assist formation of the desired bridge, forming an exendo quinone methide at the 4-position and allowing the methide to react with the 1-substituent to bring about the bridged structure. Preferred bridging reagents are of formula (XIX)



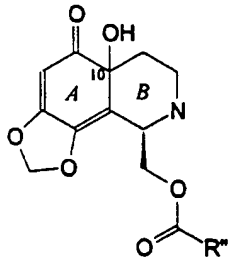

where Fu indicates a protected functional group, such as a group -NHProt$^{4a}$ or OProt$^{4b}$, Prot$^3$ is a protecting group, and the dotted line shows an optional double bond.

Suitably the methide is formed by first introducing a hydroxy group at the 10-position

at the junction of rings *A* and *B* to give a partial structure of formula (XX):




or more preferably a partial structure of formula (XXI):




where the group R" is chosen for the desired group of formula (IV), (V), (VI) or (VII). For the first two such groups, the group R" typically takes the form -CHFu-$CH_2$-SProt[3]. The protecting groups can then be removed and modified as appropriate to give the desired compound.

A typical procedure for step (d) is provided in US Patent 5,721,362 incorporated by reference. Particular reference is made to the passage at column 8, step (l) and Example 33 of the US Patent, and related passages.

Derivatisation in step (e) can include acylation, for instance with a group $R^a$-CO- as well as conversion of the 12-$NCH_3$ group to 12-NH or 12-$NCH_2CH_3$. Such conversion can be effected before or after the other steps, using available methods.

By way of illustration, it is now feasible to transform cyanosafracin B compound of formula **2** into ET-743 resulting in a shorter and more straightforward way to make ET-743 than methods previously described. Cyanosafracin B can be transformed into Intermediate **25**;

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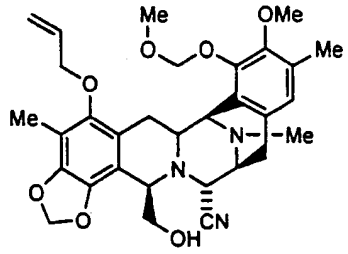
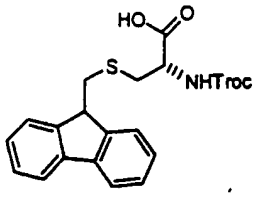
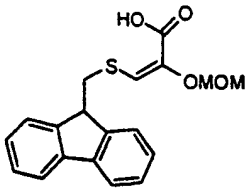


INT-25

and from this derivative it is possible to introduce a number of cysteine derivatives that can be transformed later into Et-743. Preferred cysteine derivatives are exemplified by the following two compounds:

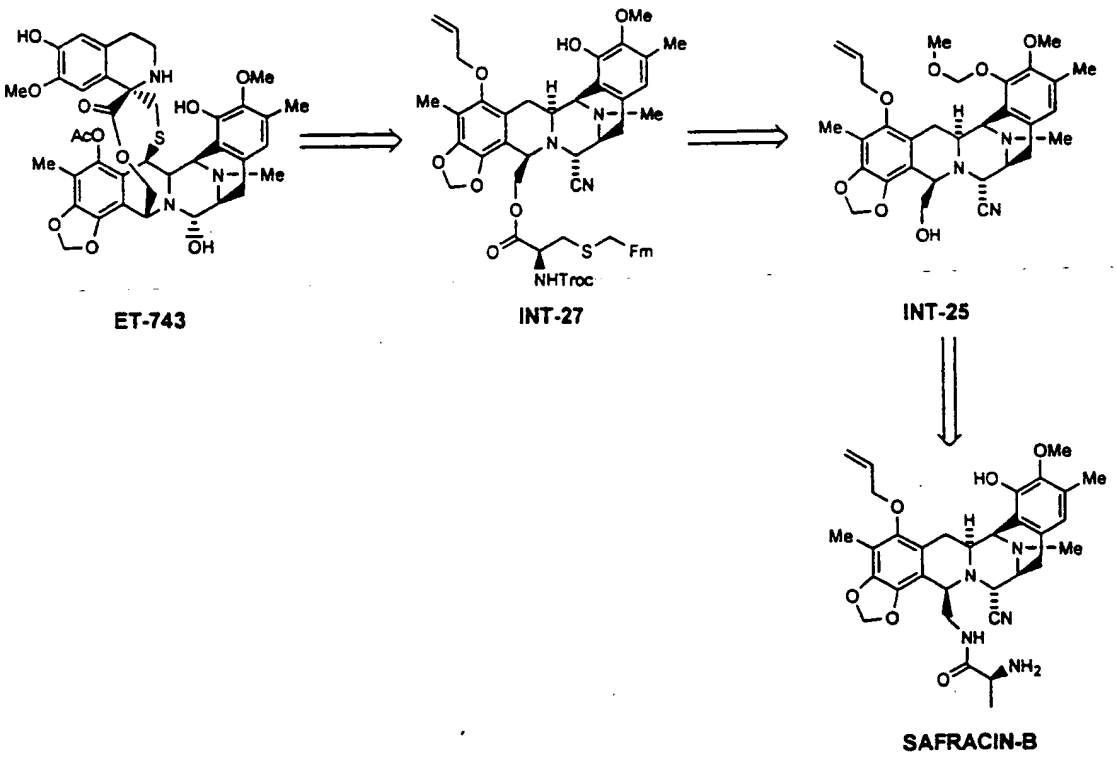


Int-29



Int-37

The retrosynthetic analysis to make ET-743 using compound **29** is depicted in scheme I.




Scheme I

Following the above scheme I it is possible to obtain ET-743 in 21 linear steps. This method transforms cyanosafracin B into intermediate **25** through a sequence of reactions that involves essentially (1) removal of methoxy group placed in ring A, (2) reduction of ring A and formation of methylene-dioxy group in one pot, (3) hydrolysis of amide function placed over carbon 1, (4) transformation of the resulting amine group into hydroxyl group. Furthermore the method avoids protection and de-protection of the primary alcohol function at the position 1 in ring B of compound **25** using directly a cysteine residue **29** to form intermediate **27**. Cysteine derivative **29** is protected in the amino group with β-β-β-trichloroethoxycarbonyl protecting group in order to have compatibility with the existing allyl and MOM groups. Intermediate **27** is directly oxidized and cycled. These circumstances, together with a different de-protecting strategy in the later stages of the synthesis makes the route novel and more amenable to industrial development than the process of US 5,721,362..

The conversion of the 2-cyano compound into Intermediate **25** usually involves the following steps (see scheme II):

formation of the protected compound of Formula **14** by reacting **2** with *tert*-butoxycarbonyl anhydride;

converting of **14** into the di-protected compound of Formula **15** by reacting with bromomethylmethyl ether and diisopropylethylamine in acetonitrile;

selectively elimination of the methoxy group of the quinone system in **15** to obtain the compound of Formula **16** by reacting with a methanolic solution of sodium hydroxide;

transforming of **16** into the methylene-dioxy compound of Formula **18** by employing the next preferred sequence: (1) quinone group of compound **16** is reduced with 10% Pd/C under hydrogen atmosphere; (2) the hydroquinone intermediate is converted into the methylenedioxy compound of Formula **17** by reacting with bromochloromethane and caesium carbonate under hydrogen atmosphere; (3) **17** is transformed into the compound of Formula **18** by protecting the free hydroxyl group as a $OCH_2R$ group. This reaction is carried out with $BrCH_2R$ and caesium carbonate, where R can be aryl, $CH{=}CH_2$, OR´ etc.

elimination of the *tert*-butoxycarbonyl and the methyloxymethyl protecting groups of **18** to afford the compound of Formula **19** by reacting with a solution of HCl in dioxane. Also this reaction is achieved by mixing **18** with a solution of trifluoroacetic acid in dichloromethane;

formation of the thiourea compound of Formula **20** by reacting **19** with phenylisothiocyanate;

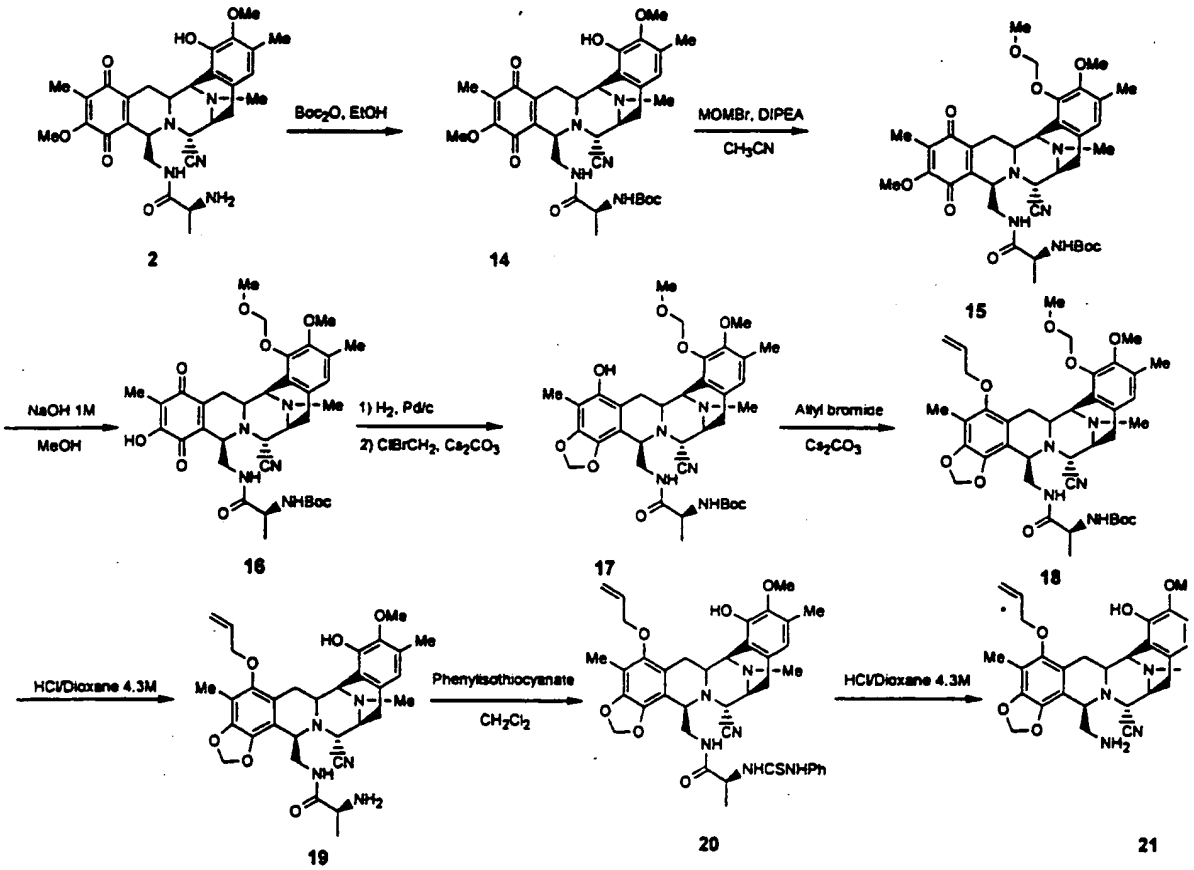
converting compound of Formula **20** into the amine compound of Formula **21** by reacting with a solution of hydrogen chloride in dioxane;

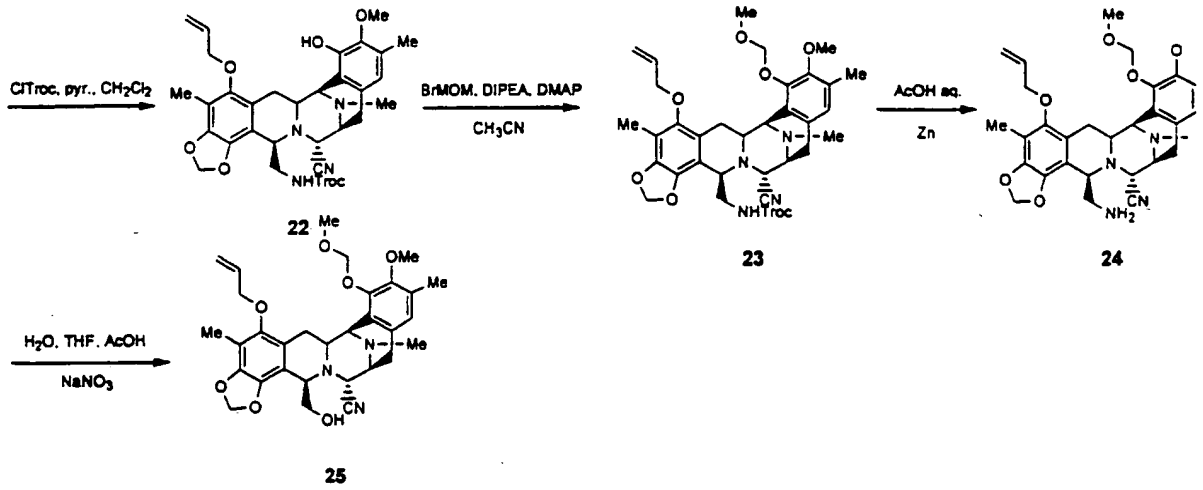
transforming compound of Formula **21** into the *N*-Troc derivative **22** by reacting with trichloroethyl chloroformate and pyridine;

formation of the protected hydroxy compound of Formula **23** by reacting **22** with bromomethylmethyl ether and diisopropylethylamine;

transforming compound of Formula **23** into the *N*-H derivative **24** by reacting with acetic acid and zinc;

conversion of compound of Formula **24** into the hydroxy compound of Formula **25** by reaction with sodium nitrite in acetic acid. Alternatively, it can be used nitrogen tetroxide in a mixture of acetic acid and acetonitrile followed by treatment with sodium hydroxide. Also, it can be used sodium nitrite in a mixture of acetic anhydride-acetic acid, followed by treatment with sodium hydroxide.






**Scheme II**

The conversion of the Intermediate **25** compound into ET-743 using cysteine derivative **29** usually involves the following steps (see scheme III):

transforming compound of formula **24** into the derivative **30** by protecting the primary hydroxyl function with (S)-N-2,2,2-tricloroethoxycarbonyl-S-(9H-fluoren-9-ylmethyl)cysteine **29**;

converting the protected compound of formula **30** into the phenol derivative **31** by cleavage of the allyl group with tributyltin hydride and dichloropalladium-bis (triphenylphosphine);

transforming the phenol compound of Formula **31** into compound of formula **32** by oxidation with benzeneseleninic anhydride at low temperature;

transforming the hydroxy compound of formula **32** into the lactone **33** by the following sequence: (1) Reacting compound of formula **32** with 2 eq. of triflic anhydride and 5 eq. of DMSO. (2) followed by reaction with 8 eq. of diisopropylethylamine. (3) followed by reaction with 4 eq of t-butyl alcohol (4) followed by reaction with 7 eq of 2-*tert*-Butyl-1,1,3,3,tetramethylguanidine (5) followed by reaction with 10 eq of acetic anhydride;

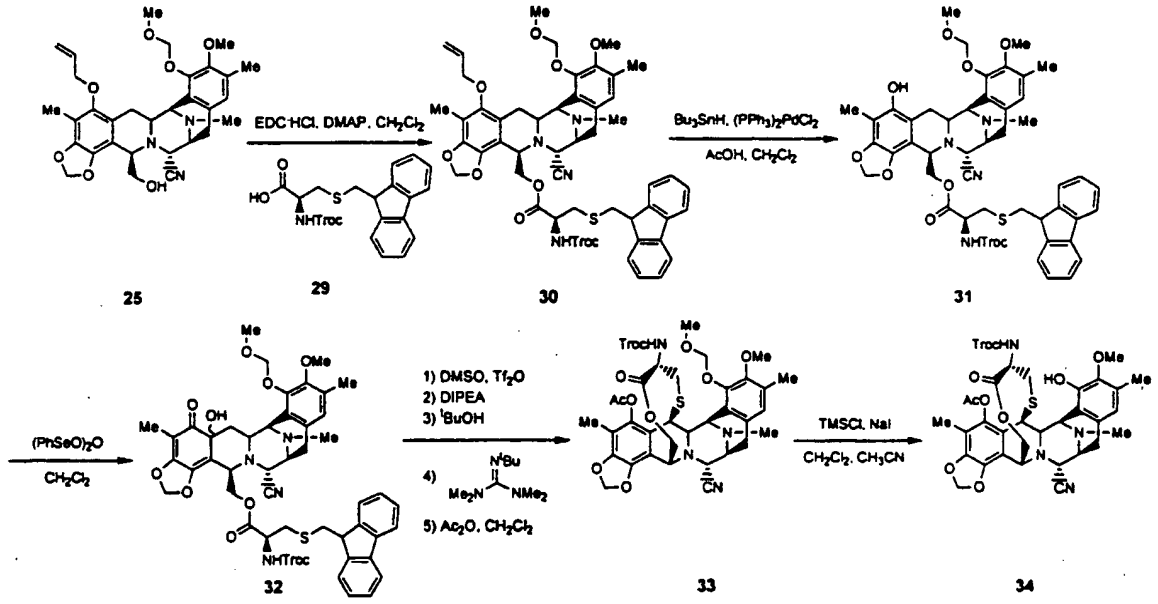
transforming the lactone compound **33** into hydroxyl compound **34** by removal of MOM protecting group with TMSI;

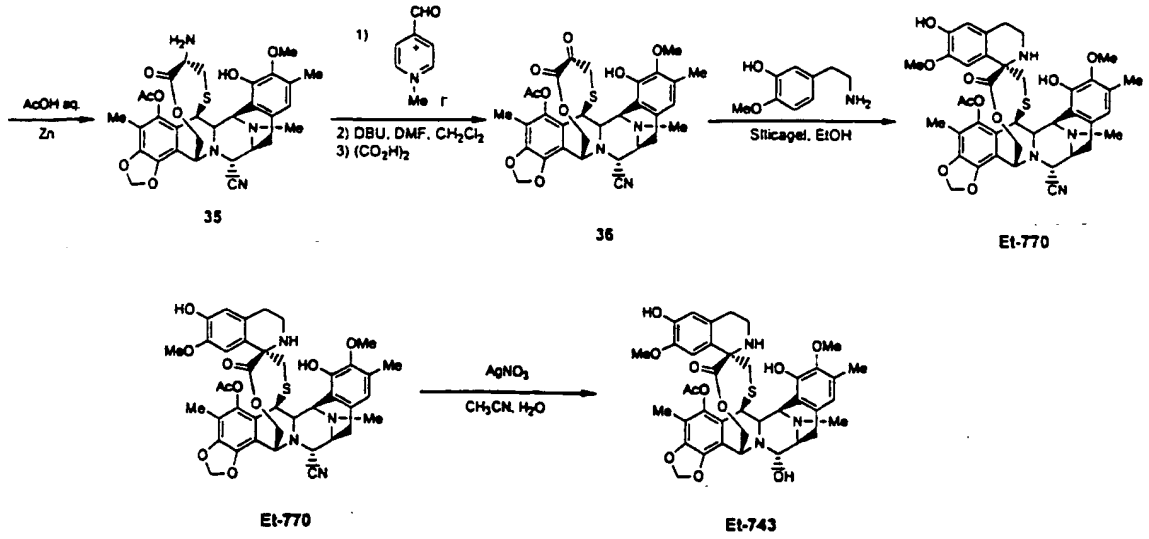
cleaving the N-trichloroethoxycarbonyl group of the compound of formula **34** into compound **35** by reaction with Zn/AcOH;

transforming the amino compound **35** into the corresponding α-keto lactone compound **36** by reaction with N-methyl pyridinium carboxaldehyde chloride followed by DBU;

forming **ET-770** by reacting compound of Formula **36** with 3-hydroxy-4-methoxyphenylethylamine;

transforming **ET-770** into **ET-743** by reaction with silver nitrate in a mixture of $AcN/H_2O$.

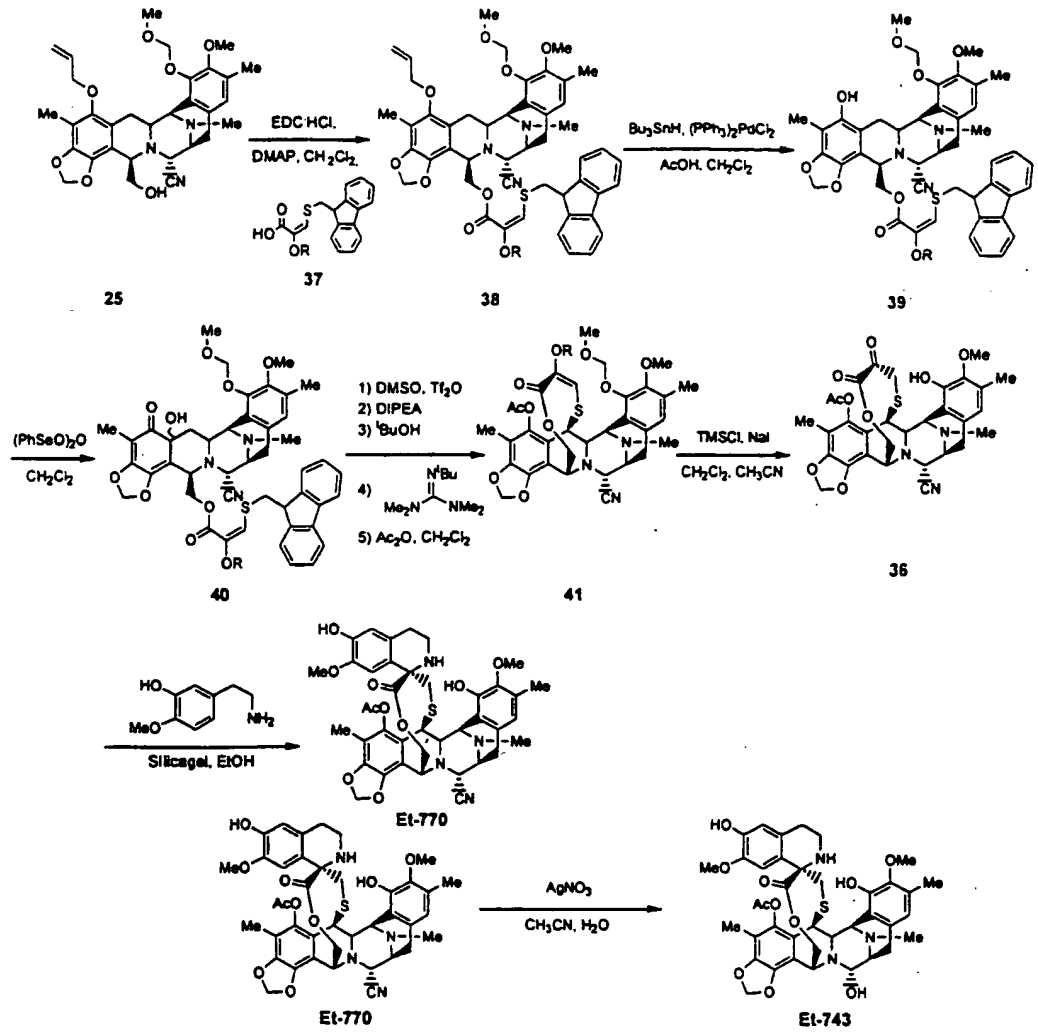





**Scheme III**

The route described above to transform Intermediate **25** into ET-743 can be conveniently modified using other cysteine derivatives, for example compound **37** named 2-methoxymethyloxy-3-(9H-fluoren-9-ylmethyl)-thio-propenoic acid. This compound has already incorporated a keto group in form of enol ether, while in the other cysteine analogs there is an amino that has to be transformed later into a keto group through a transamination reaction with a moderate yield of 55-60%. Therefore using compound **37** is possible to increase substantially the yield of the linear synthesis because the transamination step is avoided.

The conversion of the Intermediate compound **25** into ET-743 using cysteine derivative **37** can be made in a similar manner and with the same reagents than with cysteine derivative **29** with the exception of transformations (f) and (g). The reaction sequence is exemplified in the following scheme (Scheme IV):

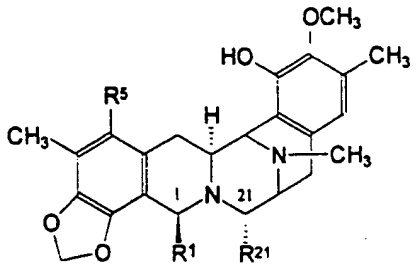



Scheme IV

Compound **38** can also be formed reacting Intermediate **12** described in U.S. patent N 5,721,362 with Intermediate **37** providing a improvement of the route described in that patent.

FORMATION OF PHTHALASCIDIN AND RELATED COMPOUNDS.

In the present invention, a key class of products includes phthalascidin and has the general formula (XX):




where $R^1$ is an amidomethylene group; $R^5$ is a small oxy-sidechain; and $R^{21}$ is a cyano group or a hydroxy group. For phthalascidin, $R^1$ is a phthalimidomethylene group; $R^5$ an acetoxy group; and $R^{21}$ is a cyano group. Other groups for $R^1$ include mono- and di-N-substituted amidomethylenes as well as other cyclic amidomethylenes, and other groups for $R^5$ include further $C_1$-$C_4$ acyl groups, as well as $C_1$-$C_4$ alkyl groups.

The conversion of the 21-cyano compound to phthalascidin or a related product of formula (XX) usually involves the following steps:

a) conversion if necessary of a quinone system for the ring *E* into the phenol system

b) formation of the -$R^5$ group at the 5-position in ring *A*;

c) formation of the $R^1$ group at the 1-position in ring *B*; and

d) conversion if necessary of a quinone system for the ring *A* into the phenol system;

e) conversion of the phenol system for the ring *A* into the methylenedioxyphenol ring.

These steps have many similarities with the steps given for formation of ecteinascidins. Step (c) typically involves forming a group -$CH_2NH_2$ at the 1-position and acylating it.

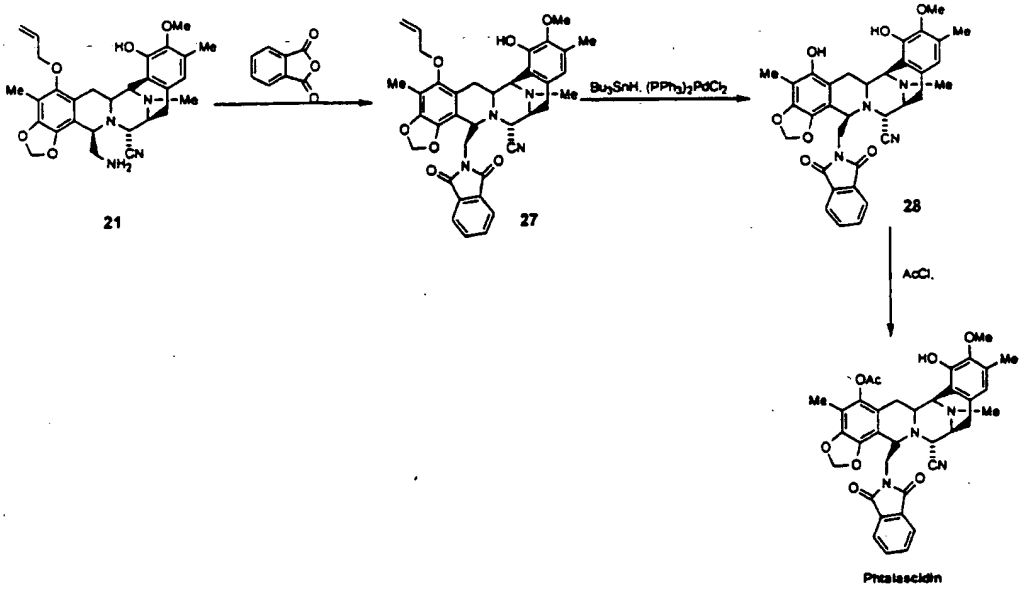
Phthlascidin can be made using Intermediates described in the conversion of cyanosafracin B into Intermediate **25**. For example, Intermediates **21** and **17** are suitable starting materials to make Phthlascidin.

As shown above in scheme V, the process for the synthetic formation of phthlascidin starting from Intermediate **21** comprises the sequential steps of:

transforming of **21** into the compound of Formula **27** by reaction with phthalic anhydride in

dichloromethane and carbonyldiimidazole.

converting of 27 into phthlascidin by reacting with tributyltin hydride and dichloro palladium-bis(triphenylphosphine) or basic media, followed by reaction with acetyl chloride.




Scheme V

As shown above in scheme VI, the process for the synthetic formation of phthlascidin starting from Intermediate **17** comprises the sequential steps of:

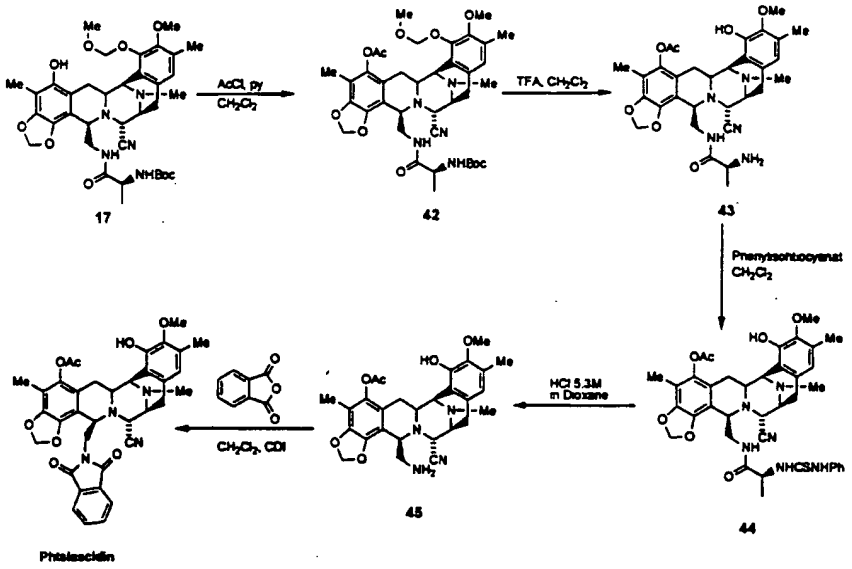
acetylation of the hydroxyl group of compound of formula **17** with acetyl chloride and pyridine to give the acetylated intermediate compound of formula **42**;

removal of the *tert*-butoxycarbonyl and the methyloxymethyl protecting groups of **42** to afford the compound of Formula **43** by reacting with a solution of HCl in dioxane. Also this reaction is achieved by mixing **42** with a solution of trifluoroacetic acid in dichloromethane;

formation of the thiourea compound of Formula **44** by reacting **43** with phenylisothiocyanate;

converting compound of Formula **44** into the amine compound of Formula **45** by reacting with a solution of hydrogen chloride in dioxane;

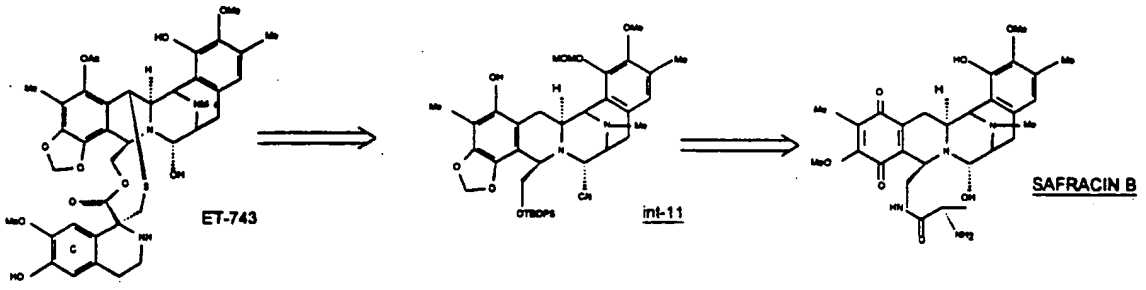
transforming of **45** into Phthlascidin by reaction with phthalic anhydride in dichloromethane and carbonyldiimidazole.



Scheme VI

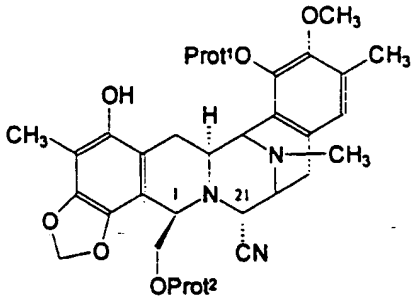
FORMATION OF INTERMEDIATE 11 AND RELATED INTERMEDIATES.

The retrosynthetic analysis is described in the following sequence.



In the present invention, a key class of intermediate includes Intermediate 11 and has

the general formula (XXI):




where $Prot^1$ and $Prot^2$ are hydroxy protecting groups, preferably different. Typically $Prot^1$ is selected from [*more generalisation needed*]. Typically $Prot^2$ is selected from [*more generalisation needed*]. For Intermediate 11 itself, the group $Prot^1$ is a methoxymethyl group, and $Prot^2$ is a t-butyldiphenylsilyl group.

The conversion of the 21-cyano compound to Intermediate 11 or a related intermediate of formula (XXI) usually involves the following steps:

a) conversion if necessary of a quinone system for the ring *E* into the phenol system

b) formation of the -$OProt^1$ group at the 18-position, in ring *E*;

c) formation of the -$CH_2$-$OProt^2$ group at the 1-position, in ring *B*; and

d) conversion if necessary of a quinone system for the ring *A* into the phenol system;

e) conversion of the phenol system for the ring *A* into the methylenedioxyphenol ring.

Step (b), formation of the -$OProt^1$ group at the 18-position in ring *E*, is a typical protection reaction for a phenol group, and no special comments need to be made. Appropriate conditions are chosen depending on the nature of the protecting group. The other steps are similar to the other reactions.

Step (b), formation of the -$CH_2$-$OProt^2$ group at the 1-position in ring *B*, is normally carried out by forming a group -$CH_2NH_2$ at the 1-position and then converting the amine function to a hydroxy function and protecting. Thus, where the starting material has a group $R^1$ which is -$CH_2$-NH-CO-$CR^{25a}R^{25b}R^{25c}$ then it is matter of removing the N-acyl group. Where the starting material has a group $R^1$ which is -$CH_2$-O-CO-R then no change may be needed for an ecteinascidin product where the substituent $R^1$ is the same. For other products,

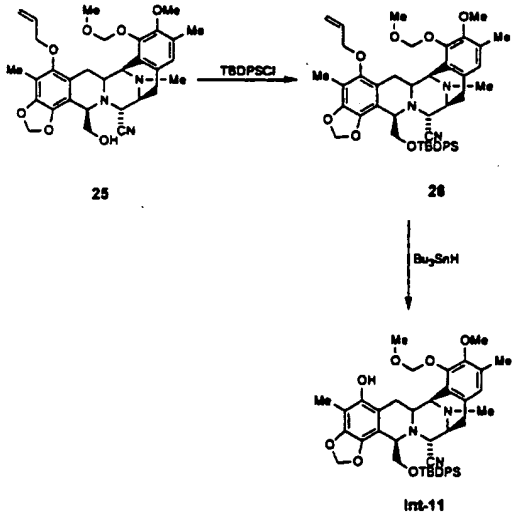
it is matter of removing the O-acyl group. Various procedures are available for such de-acylations. In one variation, the deacylation and conversion to a hydroxy function are performed in one step. Thereafter, the hydroxy group can be acylated or otherwise converted to give the appropriate $R^1$ group.

U.S. Patent N° 5,721,362 describe synthetic methods to make ET-743 through a long multistep synthesis. One of the Intermediates of this synthesis is Intermediate 11. Using cyanosafracin B as starting material it is possible to reach Intermediate 11 providing a much shorter way to make such Intermediate and therefor improving the method to make ET-743

Cyanosafracin B can be converted into Intermediate **25** by the methods described above. From Intermediate **25** is possible to reach Intermediate 11 using the following steps, see scheme VII.

formation of the protected hydroxy compound of Formula **26** by reacting **25** with *tert*-butyldiphenylsilyl chloride in the presence of a base;

final cleavage of the allyl group with tributyltin hydride and dichloropalladium-bis (triphenylphosphine) in **26** that leads to the formation of the intermediate **11.**




Scheme VII

One embodiment of the synthetic process of the present invention to transform safracin

B into intermediate **11** is a modification and extension of Scheme VIII and comprises the sequential steps of:

stereospecifically converting the compound of Formula **1** (Safracin B) to the compound of Formula **2** by selective replacement of OH by CN by reacting with KCN in acid media;

forming the thiourea compound of Formula **3** by reacting compound of Formula **2** with phenyl isothiocyanate;

converting the thiourea compound of Formula **3** into the acetamide of Formula **5** by an hydrolysis in acid media followed by addition of acetic anhydride; The intermediate amine compound of Formula **4** can be isolated by quenching the hydrolysis in acid media with sodium bicarbonate, but this intermediate is highly unstable, and is transformed quickly into a five member cyclic imine, named compound **6**;

forming the protected compound of Formula **7** by reacting with bromomethylmethyl ether and diisopropylethylamine in dichloromethane;

selectively de-methylating the methoxy group of the quinone system of compound of Formula **7** into the compound of Formula **8** by reacting with methanolic solution of sodium hydroxide;

transforming the compound of Formula **8** into methylenedioxy-compound of Formula **9** by the preferred following sequence: (1) quinone group of compound **8** is reduced with 10% Pd/C under hydrogen atmosphere; (2) the hydroquinone intermediate is converted into the methylene-dioxy compound of Formula **9** by reacting with bromochloromethane and cesium carbonate under hydrogen atmosphere; (3) compound of Formula **9** is transformed into compound of Formula **10** by protecting the free hydroxyl group as a $OCH_2R$ group, by reacting with $BrCH_2R$ and cesium carbonate, where R can be aryl, $CH{=}CH_2$, OR′ etc.;

converting the acetamide group of compound of Formula **10** into the corresponding hydroxyl group of Formula **11** by reaction with nitrogen tetroxide in a mixture of acetic acid and acetic acetate followed by treatment with sodium hydroxide; alternatively can be used sodium nitrite in a mixture of acetic anhydride acetic acid, followed by treatment with sodium hydroxide; alternatively the acetamide group of compound of Formula **10** can be converted into the primary amine group by reacting with hydrazine or with $Boc_2O$, DMAP followed by hydrazine; such primary amine can be converted into the corresponding hydroxyl group (compound of Formula **11**) by an oxidative conversion of the primary amine into the corresponding aldehyde with 4-formyl-1-methylpyridinium benzenesulphonate or other pyridinium ion, followed by DBU or other base treatment and further hydrolization, and

followed by the reduction of the aldehyde to the corresponding hydroxyl group with lithium aluminium hydride or other reducing agent;

forming the protected compound of Formula **26** by reacting with t-butyldiphenylsilyl chloride and dimethylaminopyridine in dichloromethane;

transforming the silylated compound of Formula **26** into the intermediate **11** by deprotection of the $OCH_2R$ protecting group, by reacting under reductive conditions or acid conditions. Typical procedures are with palladium black under hydrogen atmosphere, or aqueous TFA, or tributyltin hydride and dichlorobis (triphenylphosphine palladium).

In yet another preferred modification, the cyano compound of Formula **2** can be transformed into Intermediate **11** using an extension of the scheme II, involving the further steps of.

formation of the protected hydroxy compound of Formula **26** by reacting **25** with *tert*-butyldiphenylsilyl chloride in the presence of a base;

final cleavage of the allyl group with tributyltin hydride and dichloropalladium-bis (triphenylphosphine) in **26** that leads to the formation of the intermediate **11.**

FORMATION OF ACTIVE COMPOUNDS

It is possible to transform cyanosafracin B into a number of intermediates and derivatives with potential antitumor therapeutic activity. These intermediates can be made starting from already described compounds, or using alternative routes.

Intermediates described herein comprise compound **47**, and a numbers of amide derivatives made using compounds **45** or **43.**

In Scheme VIII is described formation of compound **47** using the following sequence:

forming the thiourea compound of Formula **3** by reacting compound of Formula **2** with phenyl isothiocyanate;

converting the thiourea compound of Formula **3** into the acetamide of Formula **5** by an hydrolysis in acid media followed by addition of acetic anhydride; The intermediate amine compound of Formula **4** can be isolated by quenching the hydrolysis in acid media with sodium bicarbonate, but this intermediate is highly unstable, and is transformed quickly into a five member cyclic imine, named compound **6**;

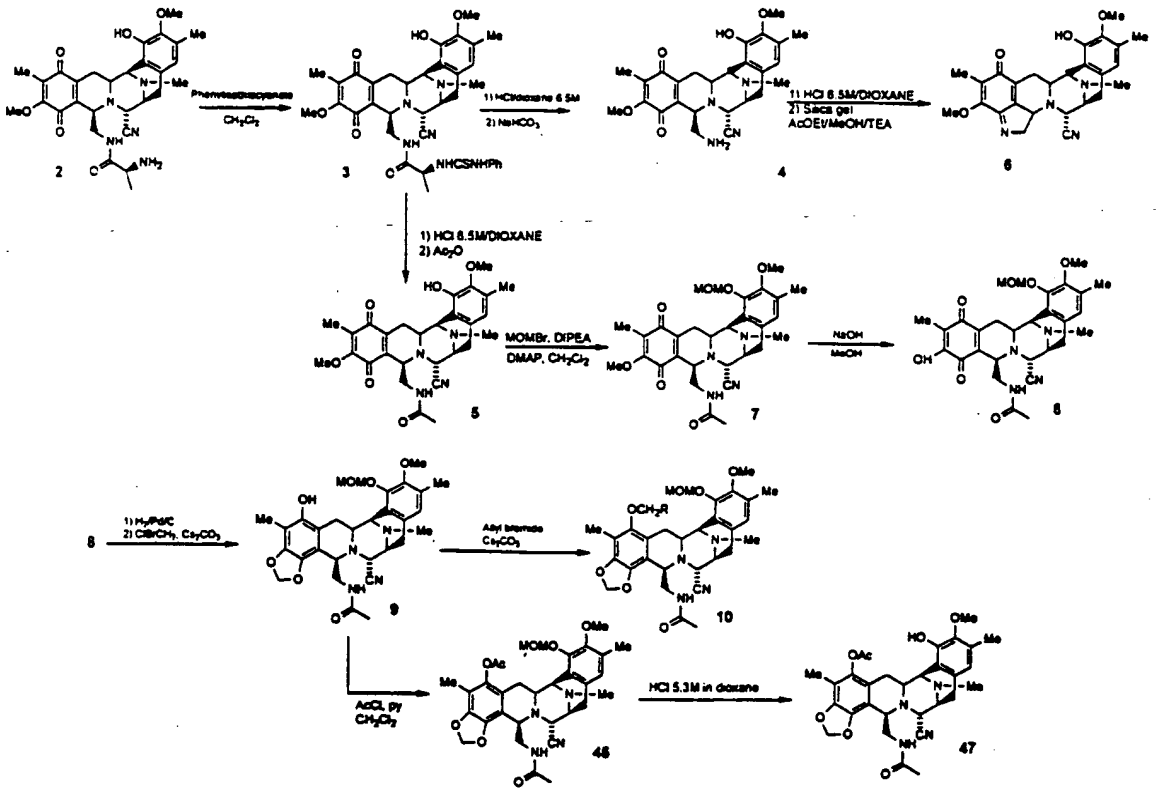
forming the protected compound of Formula **7** by reacting with bromomethylmethyl ether and diisopropylethylamine in dichloromethane;

selectively de-methylating the methoxy group of the quinone system of compound of Formula **7** into the compound of Formula **8** by reacting with methanolic solution of sodium hydroxide;

transforming the compound of Formula **8** into methylenedioxy-compound of Formula **10** by the preferred following sequence: (1) quinone group of compound **8** is reduced with 10% Pd/C under hydrogen atmosphere; (2) the hydroquinone intermediate is converted into the methylene-dioxy compound of Formula **9** by reacting with bromochloromethane and cesium carbonate under hydrogen atmosphere; (3) compound of Formula **9** is transformed into compound of Formula **10** by protecting the free hydroxyl group as a allyloxy group, by reacting with allyl-bromide and cesium carbonate;

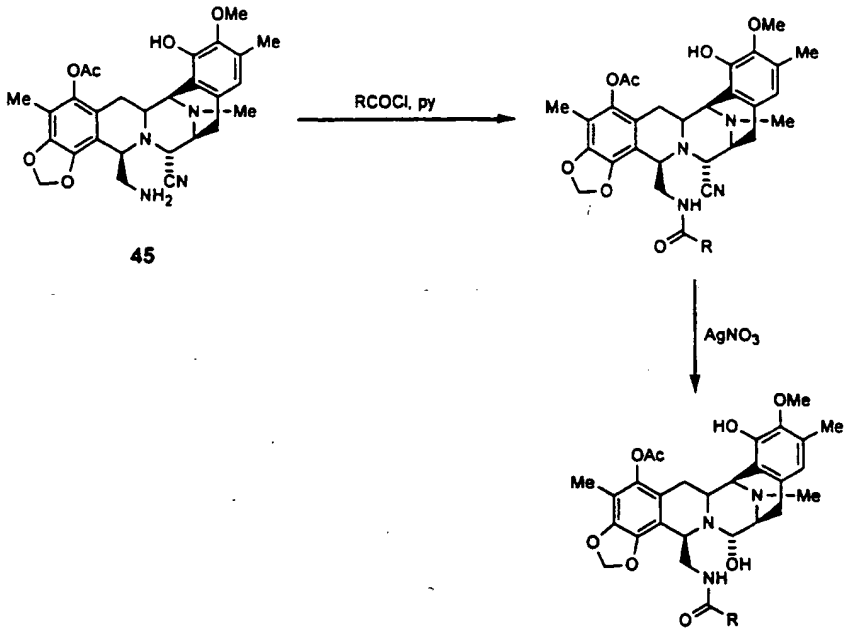
transforming the compound of formula **9** into acetyl-derivative **46** by reaction with acetyl chloride in pyridine;

transforming compound of formula **46** into de-protected compound **47** by reaction with hydrochloric acid in dioxane.



Scheme VIII

Other useful amide intermediate derivatives are made starting from already described intermediate **45** using the next scheme:




The second step is optional. This process is an important part of the invention, particularly where the group R is a group $R^a$ as previously defined. Furthermore, the Scheme VIII can be readily broadened to enable preparation of compounds of formula (XXIII), by inclusion in the starting material of a different group at the 5-position, either a group directly intended for the product or a group which can be removed or otherwise modified to give the desired group.

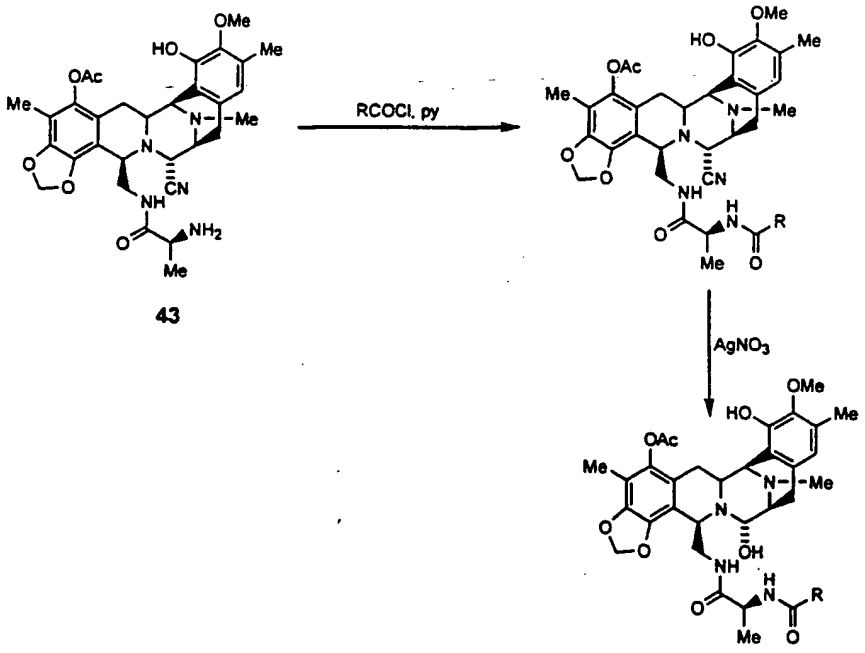
Scheme IX

From compound **45** can be made a group of analogs through the following sequence:

acylation in the amino group of compound of Formula **45** by a wide range of acyl derivatives to provide the corresponding amides, where preferred acyl groups are acetyl, cinnamoyl chloride, p-trifluorocinnamoyl chloride, isovaleryl chloride phenylisothiocyanate or aminoacids, or the other examples previously given of groups $R^aCO$-.

transforming the CN group into an OH group by reaction with silver nitrate in a mixture $AcN/H_2O$.

Other useful amide intermediate derivatives are made starting from already described intermediate **43** using the next scheme:




Scheme X

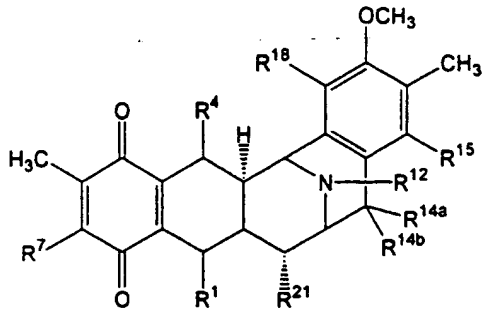
From Compound **43** can be obtained another group of interesting derivatives using the following sequence:

(a) acylation in the amino group of compound of Formula **43** by a wide range of acyl derivatives to provide the corresponding amides, where preferred acyl groups are acetyl, cinnamoyl chloride, p-trifluorocinnamoyl chloride, isovaleryl chloride or aminoacids, or the other examples previously given of groups $R^aCO$-.

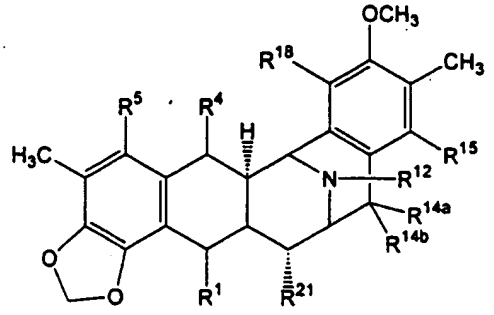
(b) transforming the CN group into an OH group by reaction with silver nitrate in a mixture $AcN/H_2O$

NOVEL INTERMEDIATE COMPOUNDS

In the light of the preceding explanations, it can be seen that the present invention provides novel intermediate compounds. Depending on ring A, the intermediates are of formula (XXIIa):



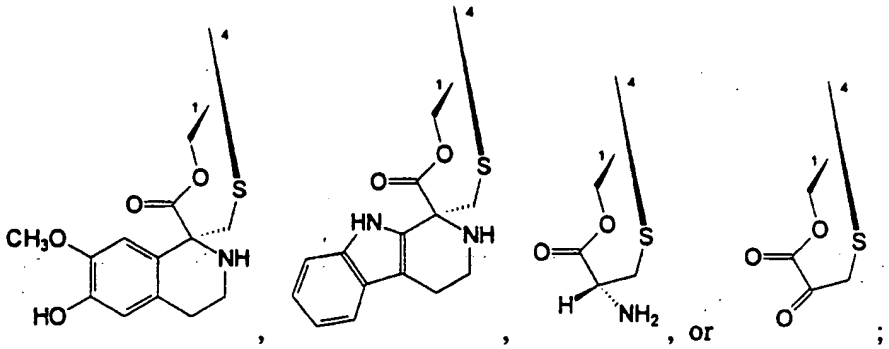
or of formula (XXIIb):



where:

$R^1$ is $-CH_2NH_2$ or $-CH_2OH$, or a protected or derivatised version of such a group and $R^4$ is -H; or

$R^{1a}$ and $R^4$ together form a group of formula (IV), (VI) or (VII):



; $R^5$ is -OH or a protected or derivatised version of such a group;

$R^{14a}$ and $R^{14b}$ are both -H or one is -H and the other is -OH or a protected or derivatised

version of such a group, $-OCH_3$ or $-OCH_2CH_3$, or $R^{14a}$ and $R^{14b}$ together form a keto group;

$R^{12}$ is -NH-, $-NCH_3-$ or $-NCH_2CH_3-$;

$R^{15}$ is -OH or a protected or derivatised version of such a group; and

$R^{18}$ is -OH or a protected or derivatised version of such a group.

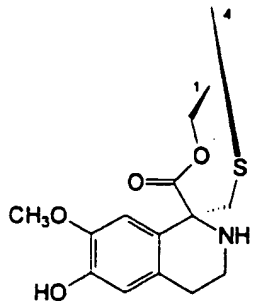
In one embodiment, preferably at least of $R^1$, $R^5$, $R^{14a}$, $R^{14b}$, $R^{15}$ or $R^{18}$ is a protected or derivatised group.

In one variation of this invention, the group $R^1$ is not a 3,5-t-butyldiphenylsilyl substituent and/or the group $R^{18}$ is not a methoxymethyl group.

Preferably $R^1$ is $-CH_2NH_2$ or $-CH_2OH$, or a protected or derivatised version of such a group and $R^4$ is -H;

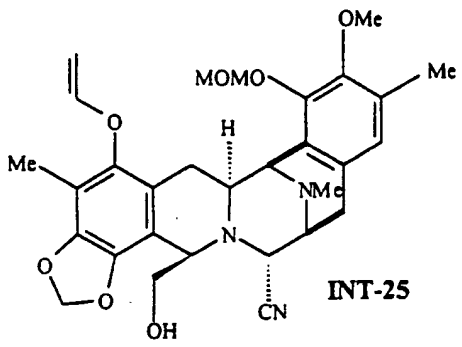
or

$R^{1a}$ and $R^4$ together form a group:




Preferably $R^{14a}$ and $R^{14b}$ are both -H.

One preferred class of intermediates includes the compound which we identify as compound **25**, of formula:




INT-25

The preferred class is thus of the general formula where the group MOM is replaced by any other protecting group.

Other preferred intermediates includes the compounds which we identify as compound **45** and **47**. Other N-acyl derivatives may readily be made from compound **45** and are an important part of this invention. Suitable acyl groups include those previously mentioned. The corresponding 21-hydroxy compounds are also useful and are among the active compounds which we have found.

## NOVEL ACTIVE COMPOUNDS

We have additionally found that certain of the compounds of the invention which we initially prepared as intermediates have exceptional activity in the treatment of cancers, such as leukaemias, lung cancer, colon cancer, kidney cancer and melanoma.

Thus, the present invention provides a method of treating any mammal, notably a human, affected by cancer which comprises administering to the affected individual a therapeutically effective amount of a compound of the invention, or a pharmaceutical composition thereof.

The present invention also relates to pharmaceutical preparations, which contain as active ingredient a compound or compounds of the invention, as well as the processes for their preparation.

Examples of pharmaceutical compositions include any solid (tablets, pills, capsules, granules, etc.) or liquid (solutions, suspensions or emulsions) with suitable composition or oral, topical or parenteral administration, and they may contain the pure compound or in combination with any carrier or other pharmacologically active compounds. These compositions may need to be sterile when administered parenterally.

Administration of the compounds or compositions of the present invention may be by any suitable method, such as intravenous infusion, oral preparations, intraperitoneal and intravenous administration. We prefer that infusion times of up to 24 hours are used, more preferably 2-12 hours, with 2-6 hours most preferred. Short infusion times which allow treatment to be carried out without an overnight stay in hospital are especially desirable. However, infusion may be 12 to 24 hours or even longer if required. Infusion may be carried out at suitable intervals of say 2 to 4 weeks. Pharmaceutical compositions containing compounds of the invention may be delivered by liposome or nanosphere encapsulation, in sustained release formulations or by other standard delivery means.

The correct dosage of the compounds will vary according to the particular formulation, the mode of application, and the particular *situs*, host and tumour being treated. Other factors like age, body weight, sex, diet, time of administration, rate of excretion, condition of the host, drug combinations, reaction sensitivities and severity of the disease shall be taken into account. Administration can be carried out continuously or periodically within the maximum tolerated dose.

The compounds and compositions of this invention may be used with other drugs to provide a combination therapy. The other drugs may form part of the same composition, or be provided as a separate composition for administration at the same time or a different time. The identity of the other drug is not particularly limited, and suitable candidates include:

a) drugs with antimitotic effects, especially those which target cytoskeletal elements, including microtubule modulators such as taxane drugs (such as taxol, paclitaxel, taxotere, docetaxel), podophylotoxins or vinca alkaloids (vincristine, vinblastine);

b) antimetabolite drugs such as 5-fluorouracil, cytarabine, gemcitabine, purine analogues

such as pentostatin, methotrexate);

c) alkylating agents such as nitrogen mustards (such as cyclophosphamide or ifosphamide);

d) drugs which target DNA such as the antracycline drugs adriamycin, doxorubicin, pharmorubicin or epirubicin;

e) drugs which target topoisomerases such as etoposide;

f) hormones and hormone agonists or antagonists such as estrogens, antiestrogens (tamoxifen and related compounds) and androgens, flutamide, leuprorelin, goserelin, cyprotrone or octreotide;

g) drugs which target signal transduction in tumour cells including antibody derivatives such as herceptin;

h) alkylating drugs such as platinum drugs (cis-platin, carbonplatin, oxaliplatin, paraplatin) or nitrosoureas;

i) drugs potentially affecting metastasis of tumours such as matrix metalloproteinase inhibitors;

j) gene therapy and antisense agents;

k) antibody therapeutics;

l) other bioactive compounds of marine origin, notably the didemnins such as aplidine;

m) steroid analogues, in particular dexamethasone;

n) anti-inflammatory drugs, in particular dexamethasone; and

o) anti-emetic drugs, in particular dexamethasone.

The present invention also extends to the compounds of the invention for use in a method of treatment, and to the use of the compounds in the preparation of a composition for treatment of cancer.

CYTOTOXIC ACTIVITY

Cell Cultures. Cells were maintained in logarithmic phase of growth in Eagle's Minimum Essential Medium, with Earle's Balanced Salts, with 2.0 mM L-glutamine, with non-essential amino acids, without sodium bicarbonate (EMEM/neaa); supplemented with

10% Fetal Calf Serum (FCS), $10^{-2}$ M sodium bicarbonate and 0.1 g/l penicillin-G + streptomycin sulfate.

A simple screening procedure has been carried out to determine and compare the antitumour activity of these compounds, using an adapted form of the method described by Bergeron et al (1984). The tumour cell line employed have been P-388 (suspension culture of a lymphoid neoplasm from DBA/2 mouse), A-549 (monolayer culture of a human lung carcinoma), HT-29 (monolayer culture of a human colon carcinoma) and MEL-28 (monolayer culture of a human melanoma).

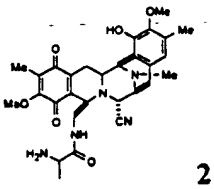
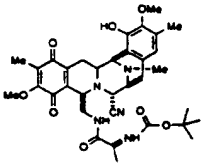
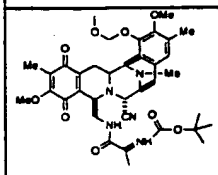
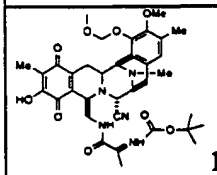
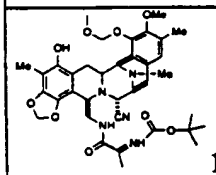
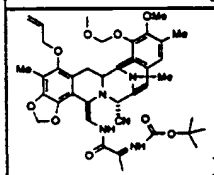
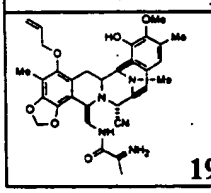
P-388 cell were seeded into 16 mm wells at $1x10^4$ cells per well in 1 ml aliquots of MEM 5FCS containing the indicated concentration of drug. A separate set of cultures without drug was seeded as control growth to ensure that cells remained in exponential phase of growth. All determinations were carried out in duplicate. After three days of incubation at 37°C, 10% $CO_2$ in a 98% humid atmosphere, an approximately $IC_{50}$ was determined by comparing the growth in wells with drug to the growth in wells control.

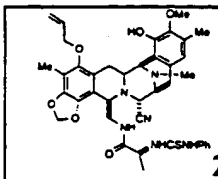
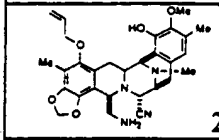
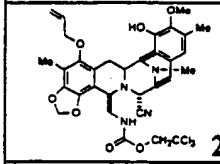
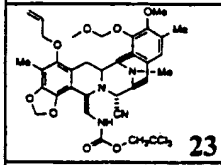
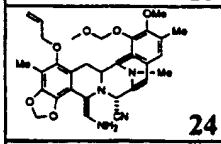
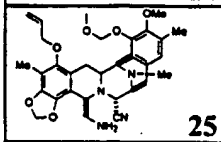
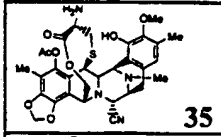
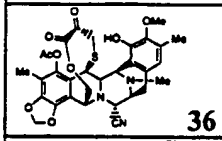
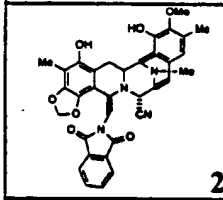
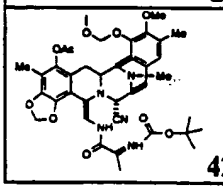
A-549, HT-29 and MEL-28 were seeded into 16 mm wells at $2x10^4$ cells per well in 1 ml aliquots of MEM 10FCS containing the indicated concentration of drug. A separate set of cultures without drug was seeded as control growth to ensure that cells remained in exponential phase of growth. All determinations were carried out in duplicate. After three days of incubation at 37°C, 10% $CO_2$ in a 98% humid atmosphere, the wells were stained with 0.1% Crystal Violet. An approximately $IC_{50}$ was determined by comparing the growth in wells with drug to the growth in wells control.

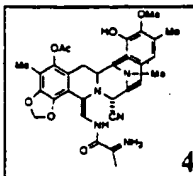
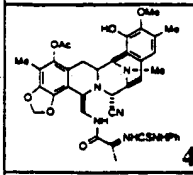
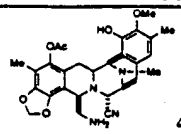
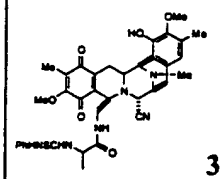
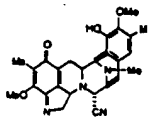
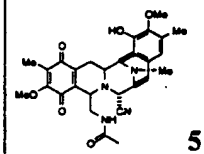
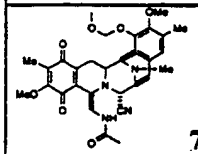
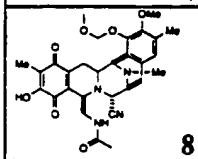
1. Raymond J. Bergeron, Paul F. Cavanaugh, Jr., Steven J. Kline. Robert G. Hughes, Jr., Gary T. Elliot and Carl W. Porter. Antineoplastic and antiherpetic activity of spermidine catecholamide iron chelators. *Biochem. Bioph. Res. Comm. 1984, 121*(3), 848-854.

2. Alan C. Schroeder, Robert G. Hughes, Jr. and Alexander Bloch. Effects of Acyclic Pyrimidine Nucleoside Analoges. *J. Med. Chem.* 1981, 24 1078-1083.

Cytotoxic activity

| Compound | $IC_{50}$ (μM) | | | | | |
|---|---|---|---|---|---|---|
| | P-388 | A-549 | HT-29 | MEL-28 | CV-1 | DU-145 |
|  2 | 0.009 | 0.018 | 0.018 | 0.018 | 0.023 | |
|  14 | 0.15 | >0.15 | 0.15 | >0.15 | | |
|  15 | 1.44 | 1.44 | 1.44 | 1.44 | | |
|  16 | >1.5 | >1.5 | >1.5 | >1.5 | | |
|  17 | 1.4 | 1.4 | 1.4 | 1.4 | | |
|  18 | 0.01 | 0.01 | 0.01 | 0.01 | | |
|  19 | 0.08 | 0.16 | 0.01 | 0.16 | | |

| | | | | | | |
|---|---|---|---|---|---|---|
|  20 | 0.01 | 0.01 | 0.01 | 0.01 | | |
|  21 | 0.019 | 0.019 | 0.019 | 0.019 | | |
|  22 | 0.014 | 0.014 | 0.014 | 0.014 | 0.014 | 0.014 |
|  23 | 0.13 | 0.13 | 0.13 | 0.13 | 0.13 | 0.13 |
|  24 | 0.18 | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 |
|  25 | 0.2 | 0.2 | 0.2 | 0.2 | | 0.2 |
|  35 | 0.008 | 0.008 | 0.008 | 0.008 | | |
|  36 | 0.01 | 0.01 | 0.01 | 0.01 | | |
|  28 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
|  42 | 0.13 | 0.13 | 0.13 | 0.13 | | 0.13 |

| | | | | | | |
|---|---|---|---|---|---|---|
|  43 | 0.008 | 0.016 | 0.008 | 0.008 | | 0.016 |
|  44 | 0.001 | 0.001 | 0.001 | 0.001 | | 0.001 |
|  45 | 0.01 | 0.01 | 0.01 | 0.01 | | 0.01 |
|  3 | 0.015 | 0.015 | 0.015 | 0.015 | 0.018 | |
|  6 | 2.171 | 2.171 | 2.171 | 2.171 | 2.171 | |
|  5 | 0.005 | 0.005 | 0.005 | 0.005 | | |
|  7 | 0.22 | 0.22 | 0.22 | 0.22 | 0.22 | |
|  8 | >9 | >18.1 | >18.1 | >18.1 | >18.1 | |

| | | | | | | |
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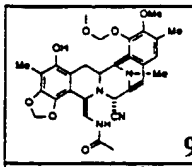
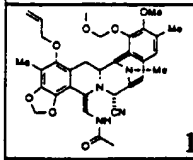
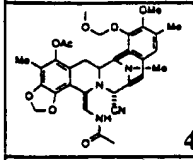
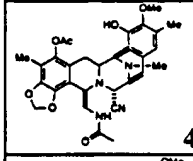
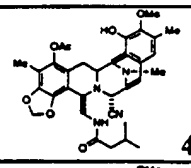
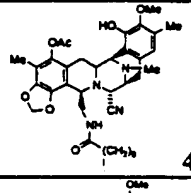
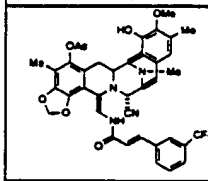
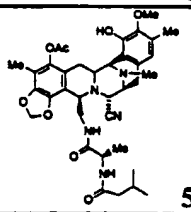
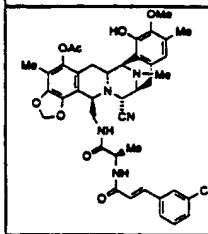
|  9 | >1.77 | >1.77 | >1.77 | >1.77 | | >1.77 |
|  10 | >1.65 | >1.65 | >1.65 | >1.65 | | >1.65 |
|  46 | 0.016 | 0.016 | 0.016 | 0.016 | | 0.016 |
|  47 | 0.001 | 0.001 | 0.001 | 0.001 | | 0.001 |
|  48 | 0.0008 | 0.001 | 0.0008 | 0.0008 | | 0.001 |
|  49 | 0.007 | 0.007 | 0.007 | 0.007 | | 0.007 |
|  50 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | | 0.0001 |
|  51 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | | 0.0001 |
|  52 | 0.001 | 0.001 | 0.001 | 0.001 | | 0.001 |

| | | | | | | |
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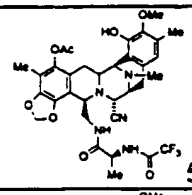
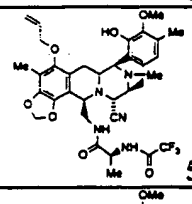
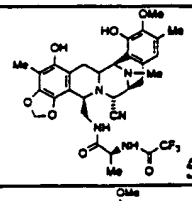
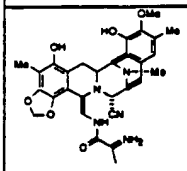
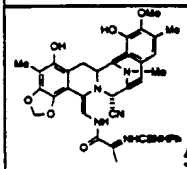
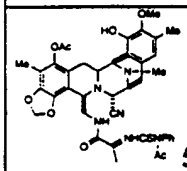
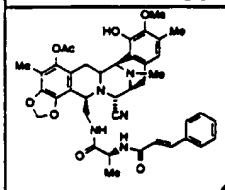
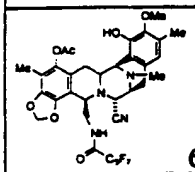
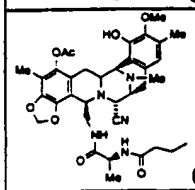
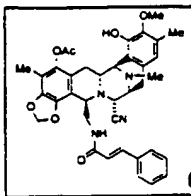
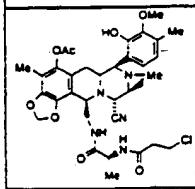
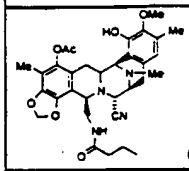
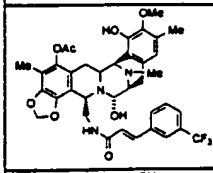
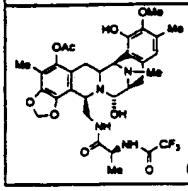
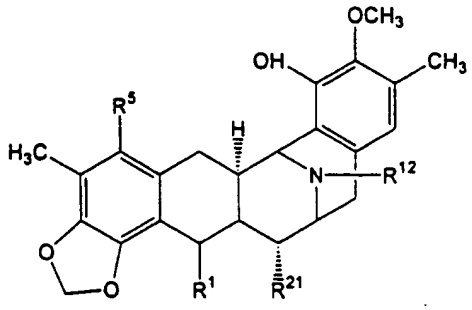
|  53 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | | 0.0001 |
|  54 | 0.001 | 0.001 | 0.001 | 0.001 | | 0.001 |
|  55 | 0.01 | 0.01 | 0.01 | 0.01 | | 0.01 |
|  56 | 0.18 | 0.9 | 0.18 | 0.8 | | 0.9 |
|  57 | 0.14 | 0.14 | 0.14 | 0.14 | | 0.14 |
|  58 | 0.001 | 0.001 | 0.001 | 0.001 | | 0.001 |
|  60 | 0.001 | 0.001 | 0.0005 | 0.001 | | 0.0005 |
|  61 | 0.001 | 0.001 | 0.001 | 0.001 | | 0.001 |
|  62 | 0.001 | 0.001 | 0.0005 | 0.0005 | | 0.001 |

| | | | | | | |
|---|---|---|---|---|---|---|
|  63 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | | 0.0001 |
|  64 | 0.001 | 0.001 | 0.001 | 0.001 | | 0.001 |
|  65 | 0.0001 | 0.0005 | 0.0001 | 0.0001 | | 0.0005 |
|  66 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | | 0.0001 |
|  67 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | | 0.0001 |

From this activity data and other considerations, it can be seen that the active compounds of this invention include a preferred class of compounds of the general formula (XXIII):




where $R^1$ is as previously defined for formula (XVIIb) and is preferably a derivatised aminomethylene group of moderate bulk;

$R^5$ is as previously defined for formula (XVIIb) and is preferably a derivatised hydroxy group of low bulk;

$R^{12}$ is as previously defined and is preferably -$NCH_3$- and

$R^{21}$ is a hydroxy or cyano group.

$R^1$ is suitably a hydrophobic group and which thus lacks free amino, hydroxy or other hydrophilic function. Typically $R^1$ is a group -$CH_2$-$NH_2$-CO-$R^a$, where $R^a$ is as defined but preferably has a linear chain length of less than 20 atoms, more preferably less than 15 or 10 atoms, where a 1,4-phenyl is counted as a chain length of four atoms and similar considerations apply to other cyclic groups (for example, 1,2-cyclohexyl is chain length of two), and the linear chain of less than 10, 15 or 20 atoms can itself be substituted. In particular, the data suggests there is a balance to be achieved between having no such group $R^a$-CO- and having a large, bulky group.

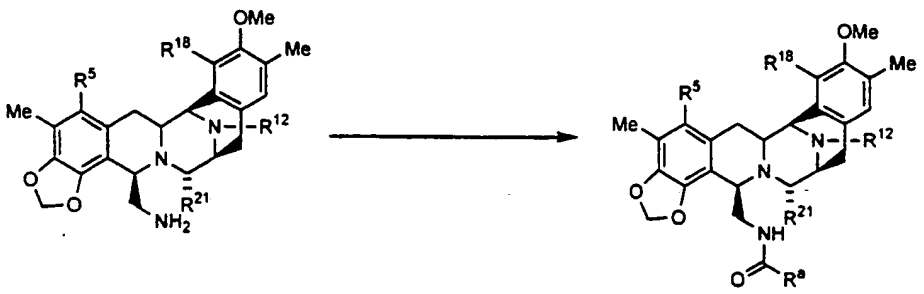
In one variation, we prefer that $R^1$ is free from cyclic groups, especially aromatic groups. In a related variation, the present invention does not prepare the compounds which are described in the article Proc. Natl. Acad. Sci. USA, 96, 3496-3501, 1999, incorporated by reference. Our preferred groups for $R^1$ exclude the corresponding substituents $CH_2R_2$ shown in Table 1 of that article, specifically the groups A, B, C and D for $R_2$.

$R^5$ is preferably an acetyl group.

In particularly preferred compounds, the group $R^1$ is acylated on an -$NH_2$ group, and for example N-acyl derivatives can be formed from groups -$CH_2NH_2$ and -$CH_2$-NH-aa. The acyl derivatives can be N-acyl or N-thioacyl derivatives thereof. The acyl groups can be of formula -CO-$R^a$, where $R^a$ is as defined and is chosen to meet the indicated criteria. Suitable acyl groups include alanyl, arginyl, aspartyl, asparagyl, cystyl, glutamyl, glutaminyl, glycyl, histidyl, hydroxyprolyl., isoleucyl, leucyl, lysyl, methionyl, phenylalanyl, prolyl, seryl, threonyl, thyronyl, tryptophyl, tyrosyl, valyl, as well as other amino acid acyl groups. Such amino acid acyl groups are preferred derivatised on the amino group to give hydrophobicity.

In a variation, the group $R^1$ is a derivatised hydroxymethylene group. Similar considerations apply as with the derivatised aminomethylene group.

Reflecting the active compounds, an important process in accordance with this invention is as follows:



where $R^5$ for the end product is as defined for the compound (XXXII) and may be different in the starting material and converted thereto as part of the process,

$R^{18}$ is a hydroxy group in the end product but may be a protected hydroxy group in the starting material and converted thereto as part of the process,

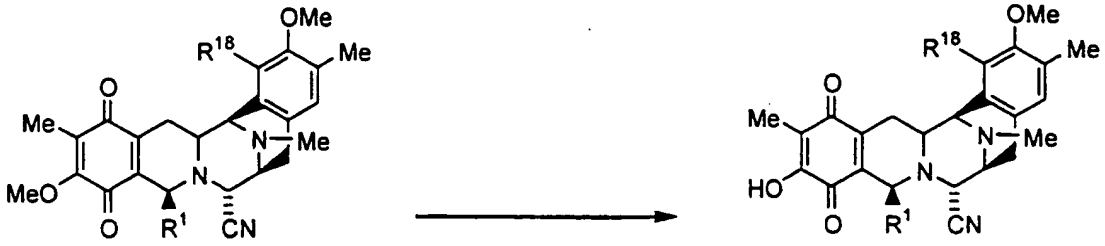
$R^{12}$ for the end product may be the same as in the starting material or may be converted thereto as part of the process,

$R^{21}$ for the end product is as defined and if a hydroxy group may be formed from a cyano group as part of the process,

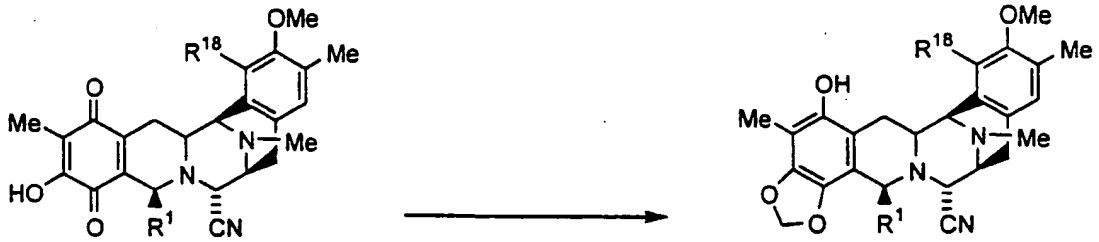
$R^a$ is as defined, and may be further acylated as part of the process to give an end product with an acylated $R^a$ group as discussed.

$R^5$ is preferably acetyl or other small acyl group in the starting material and is not changed in the reaction. $R^{18}$ is preferably a hydroxy group in the starting material and is not changed in the reaction. $R^{12}$ is preferably -$NCH_3$- in the starting material and is not changed in the reaction. $R^{21}$ the end product is as defined and if a hydroxy group may be formed from a cyano group as part of the process. $R^a$ is in the final product is preferably as defined in relation to the compound of formula (XXIII).

Another important method of this invention includes the reaction:

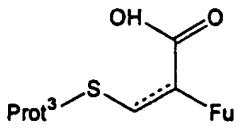


Another important method of this invention includes the reaction:



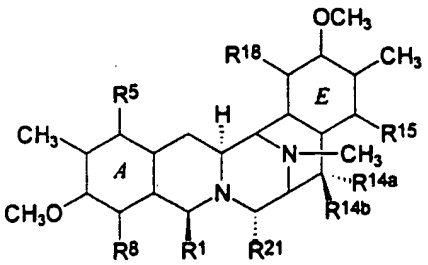
Another important method of this invention includes the reaction includes the reaction where a group $R^1$ is aminomethylene is converted to a hydroxymethylene group.

Another important method of this invention includes the reaction wherein a compound with a group $R^1$ which is hydroxymethylene is reacted with a reagent of the formula (XIX)



where Fu indicates a protected functional group, $Prot^3$ is a protecting group, and the dotted line shows an optional double bond.

Another important method of this invention includes the reaction for preparing a 21-cyano compound of formula (XVI) which comprises reacting a compound of formula (XV):



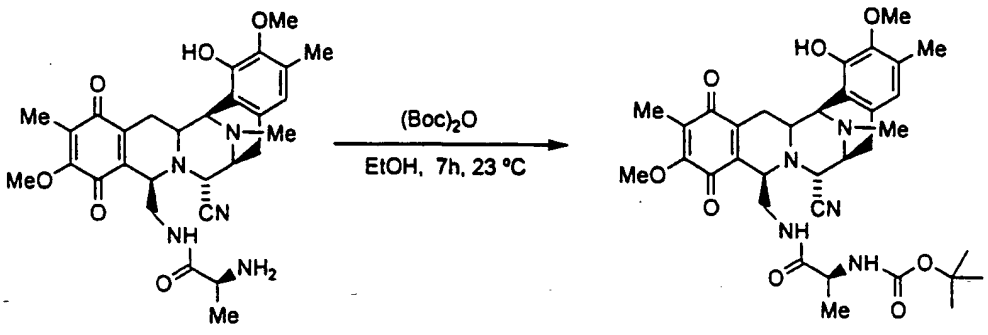
where $R^1$, $R^5$, $R^8$, $R^{14a}$, $R^{14b}$, $R^{15}$ and $R^{18}$ are as defined and $R^{21}$ is a hydroxy group, with a source of cyanide ion, to give the desired 21-cyano compound.

In addition, processes using other nucleophile-containing compounds, to produce similar compounds of formula (XVI) wherein the 21-position is protected by another nucleophilic group, a 21-Nuc group, are also envisaged. For example, a 21-Nuc compound of formula (XVI) with an alkylamino substituent at the 21-position can be produced by reacting the compound of formula (XV) wherein $R^{21}$ is a hydroxy group with a suitable alkylamine. A 21-Nuc compound of formula (XVI) with an alkylthio substituent at the 21-position can also be produced by reacting the compound of formula (XV) wherein $R^{21}$ is a hydroxy group with a suitable alkanethiol. Alternatively, a 21-Nuc compound of formula (XVI) with an α-carbonylalkyl substituent at the 21-position can be produced by reacting the compound of formula (XV) wherein $R^{21}$ is a hydroxy group with a suitable carbonyl compound, typically in the presence of a base. Other synthetic routes are available for other 21-Nuc compounds.

Another important reaction of this invention involves treatment of a 21-cyano product of this invention to form a 21-hydroxy compound. Such compounds have interesting *in vivo* properties.

EXAMPLES

Example 1




2 14

To a solution of **2** (21.53 g, 39.17 ml) in ethanol (200 ml), *tert*-butoxycarbonyl anhydride (7.7 g, 35.25 ml) was added and the mixture was stirred for 7 h at 23 °C. Then, the reaction was concentrated *in vacuo* and the residue was purified by flash column chromatography ($SiO_2$, hexane:ethyl acetate 6:4 ) to give **14** (20.6 g, 81 %) as a yellow solid.

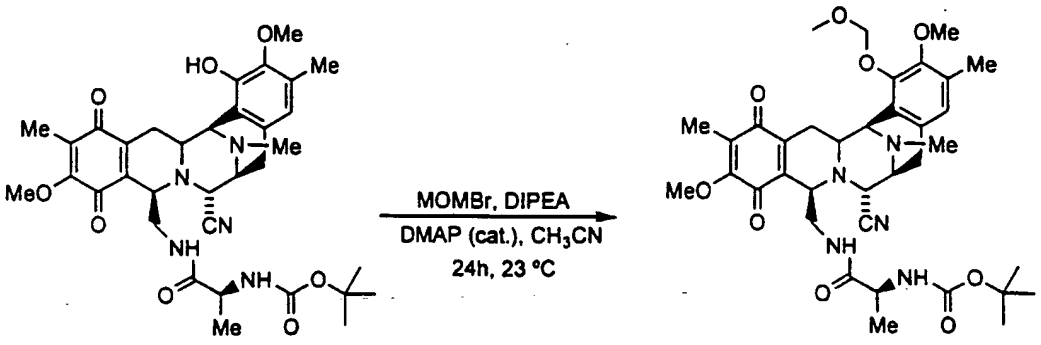
Rf: 0.52 (ethyl acetate:$CHCl_3$ 5:2).

$^1$H NMR (300 MHz, $CDCl_3$): δ 6.49 (s, 1H), 6. 32 (bs, 1H), 5.26 (bs, 1H), 4.60 (bs, 1H), 4.14 (d, *J*= 2.4 Hz, 1H), 4.05 (d, *J*= 2.4 Hz, 1H), 3.94 (s, 3H), 3.81 (d, *J*= 4.8 Hz, 1H), 3.7 (s, 3H), 3.34 (br d, *J*= 7.2 Hz, 1H), 3.18-3.00 (m, 5H), 2.44 (d, *J*= 18.3 Hz, 1H), 2.29 (s, 3H), 2.24 (s, 3H), 1.82 (s, 3H), 1.80-1.65 (m, 1H), 1.48 (s, 9H), 0.86 (d, *J*= 5.7 Hz, 3H)

$^{13}$C NMR (75 MHz, $CDCl_3$): δ 185.5, 180.8, 172.7, 155.9, 154.5, 147.3, 143.3, 141.5, 135.3, 130.4, 129.2, 127.5, 120.2, 117.4, 116.9, 80.2, 60.7, 60.3, 58.5, 55.9, 55.8, 54.9, 54.4, 50.0, 41.6, 40.3, 28.0, 25.3, 24.0, 18.1, 15.6, 8.5.

ESI-MS m/z: Calcd. for $C_{34}H_{43}N_5O_8$: 649.7. Found $(M+H)^+$: 650.3.

Example 2




14 15

To a stirred solution of **14** (20.6 g, 31.75 ml) in $CH_3CN$ (159 ml), diisopropylethylamine (82.96 ml, 476.2 ml), methoxymethylene bromide (25.9 ml, 317.5 ml) and dimethylaminopyridine (155 mg, 1.27 ml) were added at 0 °C. The mixture was stirred at 23 °C for 24h. The reaction was quenched at 0 °C with aqueous 0.1N HCl (750 ml) (pH = 5), and extracted with $CH_2Cl_2$ (2 x 400 ml). The organic phase was dried (sodium sulphate) and concentrated *in vacuo*. The residue was purified by flash column chromatography ($SiO_2$, gradient hexane:ethyl acetate 4:1 to hexane:ethyl acetate 3:2) to give **15** (17.6 g, 83 %) as a yellow solid.

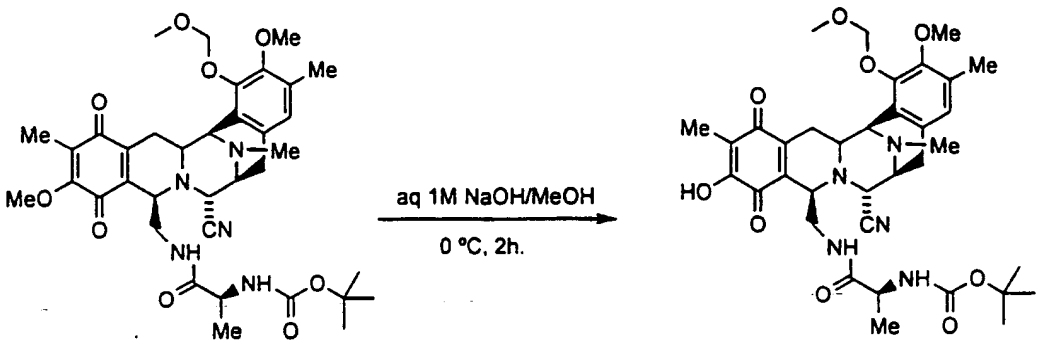
Rf: 0.38 (hexane:ethyl acetate 3:7).

$^1$H NMR (300 MHz, $CDCl_3$): δ 6.73 (s, 1H), 5.35 (bs, 1H), 5.13 (s, 2H), 4.50 (bs, 1H), 4.25 (d, *J*= 2.7 Hz, 1H), 4.03 (d, *J*= 2.7 Hz, 1H), 3.97 (s, 3H), 3.84 (bs, 1H), 3.82-3.65 (m, 1H), 3.69 (s, 3H), 3.56 (s, 3H), 3.39-3.37 (m, 1H), 3.20-3.00 (m, 5H), 2.46 (d, *J*= 18 Hz, 1H), 2.33 (s, 3H), 2.23 (s, 3H), 1.85 (s, 3H), 1.73-1.63 (m, 1H), 1.29 (s, 9H), 0.93 (d, *J*= 5.1 Hz, 3H)

$^{13}$C NMR (75 MHz, $CDCl_3$): δ 185.4, 180.9, 172.4, 155.9, 154.5, 149.0, 148.4, 141.6, 135.1, 131.0, 129.9, 127.6, 124.4, 123.7, 117.3, 99.1, 79.3, 60.7, 59.7, 58.4, 57.5, 56.2, 55.9, 55.0, 54.2, 50.0, 41.5, 39.9, 28.0, 25.2, 24.0, 18.1, 15.6, 8.5.

ESI-MS m/z: Calcd. for $C_{36}H_{47}N_5O_9$: 693.8. Found $(M+H)^+$: 694.3.

Example 3




15 16

To a flask containing **15** (8 g, 1.5 ml) in methanol (1.6 l) an aqueous solution of 1M sodium hydroxide (3.2 l) was added at 0 °C. The reaction was stirred for 2h at this temperature and then, quenched with 6M HCl to pH = 5. The mixture was extracted with ethyl acetate (3 x 1 l) and the combined organic layers were dried over sodium sulphate and concentrated *in vacuo*. The residue was purified by flash column chromatography ($SiO_2$, gradient $CHCl_3$ to $CHCl_3$:ethyl acetate 2:1) to afford **16** (5.3 mg, 68 %).

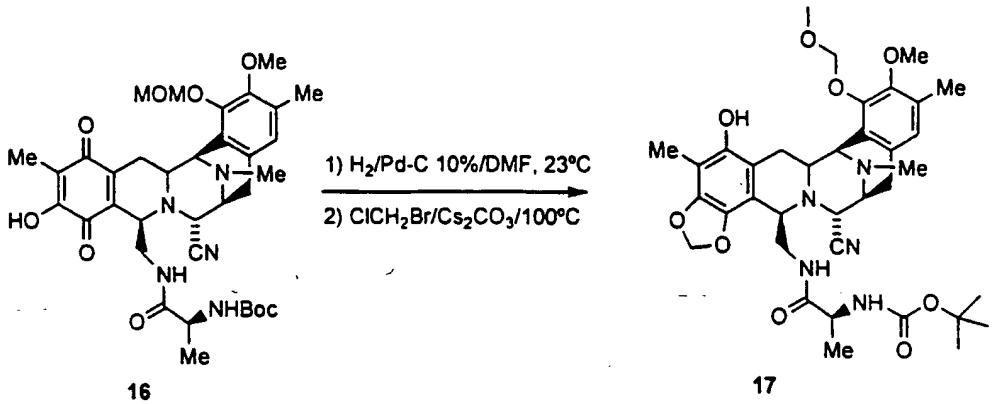
Rf: 0.48 ($CH_3CN$:$H_2O$ 7:3, RP-C18)

$^1H$ NMR (300 MHz, $CDCl_3$): δ 6.73 (s, 1H), 5.43 (bs, 1H), 5.16 (s, 2H), 4.54 (bs, 1H), 4.26 (d, *J*= 1.8 Hz, 1H), 4.04 (d, *J*= 2.7 Hz 1H), 3.84 (bs, 1H), 3.80-3.64 (m, 1H), 3.58 (s, 3H), 3.41-3.39 (m, 1H), 3.22-3.06 (m, 5H), 2.49 (d, *J*= 18.6 Hz 1H), 2.35 (s, 3H), 2.30-2.25 (m, 1H), 2.24 (s, 3H), 1.87 (s, 3H), 1.45-1.33 (m, 1H), 1.19 (s, 9H), 1.00 (br d, *J*= 6.6 Hz 3H)

$^{13}C$ NMR (75 MHz, $CDCl_3$): δ 184.9, 180.9, 172.6, 154.7, 151.3, 149.1, 148.6, 144.7, 132.9, 131.3, 129.8, 124.5, 123.7, 117.3, 116.8, 99.1, 79.4, 59.8, 58.6, 57.7, 56.2, 55.6, 54.9, 54.5, 50.1, 41.6, 40.1, 28.0, 25.3, 24.4, 18.1, 15.7, 8.0.

ESI-MS m/z: Calcd. for $C_{35}H_{45}N_5O_9$: 679.7. Found $(M+H)^+$: 680.3.

Example 4




16 17

To a degassed solution of compound **16** (1.8 g, 2.64 ml) in DMF (221 ml) 10.% Pd/C (360 mg) was added and stirred under $H_2$ (atmospheric pressure) for 45 min. The reaction was filtered through celite under argon, to a flask containing anhydrous $Cs_2CO_3$ (2.58 g, 7.92 ml). Then, bromochloromethane (3.40 ml 52.8 ml), was added and the tube was sealed and stirred at 100 °C for 2h. The reaction was cooled, filtered through a pad of celite and washed with $CH_2Cl_2$. The organic layer was concentrated and dried (sodium sulphate) to afford **17** as a brown oil that was used in the next step with no further purification.

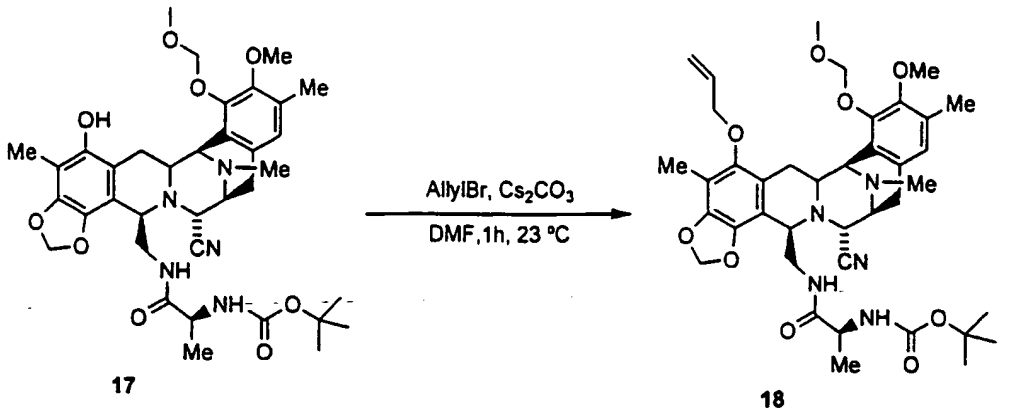
Rf: 0.36 (hexane:ethyl acetate 1:5, $SiO_2$).

$^1$H NMR (300 MHz, $CDCl_3$): δ 6.68 (s, 1H), 6.05 (bs, 1H), 5.90 (s, 1H), 5.79 (s, 1H), 5.40 (bs, 1H), 5.31-5.24 (m, 2H), 4.67 (d, *J*= 8.1 Hz, 1H), 4.19 (d, *J*= 2.7 Hz, 1H), 4.07 (bs, 1H), 4.01 (bs, 1H), 3.70 (s, 3H), 3.67 (s, 3H), 3.64-2.96 (m, 5H), 2.65 (d, *J*=18.3 Hz, 1H), 2.33 (s, 3H), 2.21 (s, 3H), 2.04 (s, 3H), 2.01-1.95 (m, 1H), 1.28 (s, 9H), 0.87 (d, *J*= 6.3 Hz, 3H)

$^{13}$C NMR (75 MHz, $CDCl_3$): δ 172.1, 162.6, 154.9, 149.1, 145.7, 135.9, 130.8, 130.7, 125.1, 123.1, 117.8, 100.8, 99.8, 76.6, 59.8, 59.2, 57.7, 57.0, 56.7, 55.8, 55.2, 49.5, 41.6, 40.1, 36.5, 31.9, 31.6, 29.7, 28.2, 26.3, 25.0, 22.6, 18.2, 15.8, 14.1, 8.8.

ESI-MS m/z: Calcd. for $C_{36}H_{47}N_5O_9$: 693.34. Found $(M+H)^+$: 694.3.

Example 5




17 18

To a flask containing a solution of **17** (1.83 g, 2.65 ml) in DMF (13 ml), $Cs_2CO_3$ (2.6 g, 7.97 ml), and allyl bromide (1.15 ml, 13.28 ml) were added at 0° C. The resulting mixture was stirred at 23 °C for 1h. The reaction was filtered through a pad of celite and washed with $CH_2Cl_2$. The organic layer was dried and concentrated (sodium sulphate). The residue was purified by flash column chromatography ($SiO_2$, $CHCl_3$:ethyl acetate 1:4) to afford **18** (1.08 mg, 56 %) as a white solid.

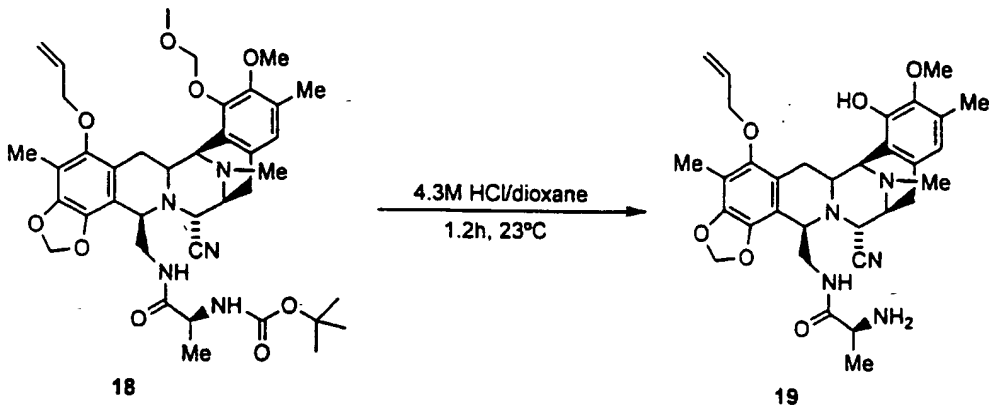
Rf: 0.36 ($CHCl_3$:ethyl acetate 1:3).

$^1$H NMR (300 MHz, $CDCl_3$): δ 6.70 (s, 1H), 6.27-6.02 (m, 1H), 5.94 (s, 1H), 5.83 (s, 1H), 5.37 (dd, $J_1$= 1.01 Hz, $J_2$= 16.8 Hz, 1H), 5.40 (bs, 1H), 5.25 (dd, $J_1$= 1.0 Hz, $J_2$= 10.5 Hz, 1H), 5.10 (s, 2H), 4.91 (bs, 1H), 4.25-4.22 (m, 1H), 4.21 (d, $J$= 2.4 Hz, 1H), 4.14-4.10 (m, 1H), 4.08 (d, $J$=2.4 Hz, 1H), 4.00 (bs, 1H), 3.70 (s, 3H), 3.59 (s, 3H), 3.56-3.35 (m, 2H), 3.26-3.20 (m, 2H), 3.05-2.96 (dd, $J_1$= 8.1 Hz, $J_2$=18 Hz, 1H), 2.63 (d, $J$=18 Hz, 1H), 2.30 (s, 3H), 2.21 (s, 3H), 2.09 (s, 3H), 1.91-1.80 (m, 1H), 1.24 (s, 9H), 0.94 (d, $J$= 6.6 Hz, 3H)

$^{13}$C NMR (75 MHz, $CDCl_3$): δ 172.0, 154.8, 148.8, 148.6, 148.4, 144.4, 138.8, 133.7, 130.9, 130.3, 125.1, 124.0, 120.9, 117.8, 117.4, 112.8, 112.6, 101.1, 99.2, 73.9, 59.7, 59.3, 57.7, 56.9, 56.8, 56.2, 55.2, 40.1, 34.6, 31.5, 28.1, 26.4, 25.1, 22.6, 18.5, 15.7, 14.0, 9.2.

ESI-MS m/z: Calcd. for $C_{39}H_{51}N_5O_9$: 733.4. Found $(M+H)^+$: 734.4.

Example 6




18 19

To a solution of **18** (0.1 g, 0.137 ml) in dioxane (2 ml), 4.2M HCl/dioxane (1.46 ml) was added and the mixture was stirred for 1.2h at 23 °C. The reaction was quenched at 0 °C with sat. Aqueous sodium bicarbonate (60 ml) and extracted with ethyl acetate (2x70 ml). The organic layers were dried (sodium sulphate) and concentrated *in vacuo* to afford **19** (267 mg, 95 %) as a white solid that was used in subsequent reactions with no further purification.

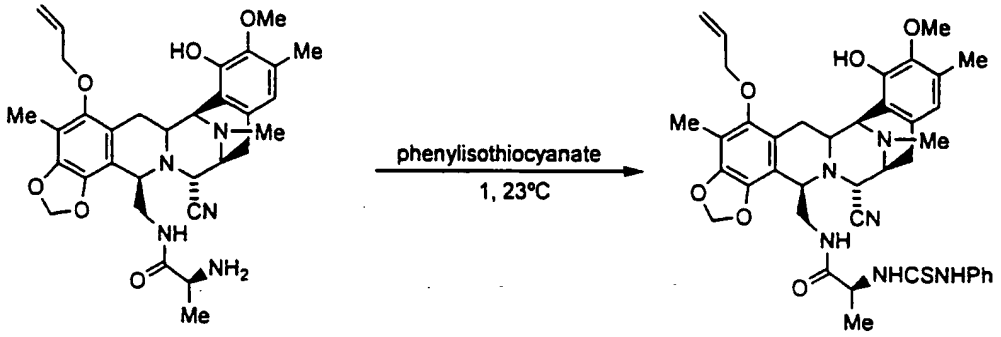
Rf: 0.17 (ethyl acetate:methanol 10:1, $SiO_2$)

$^1$H NMR (300 MHz, $CDCl_3$): δ 6.49 (s, 1H), 6.12-6.00 (m, 1H), 5.94 (s, 1H), 5.86 (s, 1H), 5.34 (dd, *J*= 1.0 Hz, *J*= 17.4 Hz, 1H), 5.25 (dd, *J*= 1.0 Hz, *J*= 10.2 Hz, 1H), 4.18-3.76 (m, 5H), 3.74 (s, 3H), 3.71-3.59 (m, 1H), 3.36-3.20 (m, 4H), 3.01-2.90 (m, 1H), 2.60 (d, *J*= 18.0 Hz, 1H), 2.29 (s, 3H), 2.24 (s, 3H), 2.11 (s, 3H), 1.97-1.86 (m, 1H), 0.93 (d, *J*= 8.7 Hz, 3H)

$^{13}$C NMR (75 MHz, $CDCl_3$): δ 175.5, 148.4, 146.7, 144.4, 142.4, 138.9, 133.7, 131.3, 128.3, 120.8, 117.9, 117.4, 113.8, 112.4, 101.1, 74.2, 60.5, 59.1, 56.5, 56.1, 56.3, 56.0, 55.0, 50.5, 41.6, 39.5, 29.5, 26.4, 24.9, 21.1, 15.5, 9.33.

ESI-MS m/z: Calcd. for $C_{32}H_{39}N_5O_6$: 589. Found $(M+H)^+$: 590.

Example 7




To a solution of **19** (250 mg, 0.42 ml) in $CH_2Cl_2$ (1.5 ml), phenyl isothiocyanate (0.3 ml, 2.51 ml) was added and the mixture was stirred at 23° C for 1h. The reaction was concentrated *in vacuo* and the residue was purified by flash column chromatography ($SiO_2$, gradient Hexane to 5:1 hexane:ethyl acetate) to afford **20** (270 mg, 87 %) as a white solid.

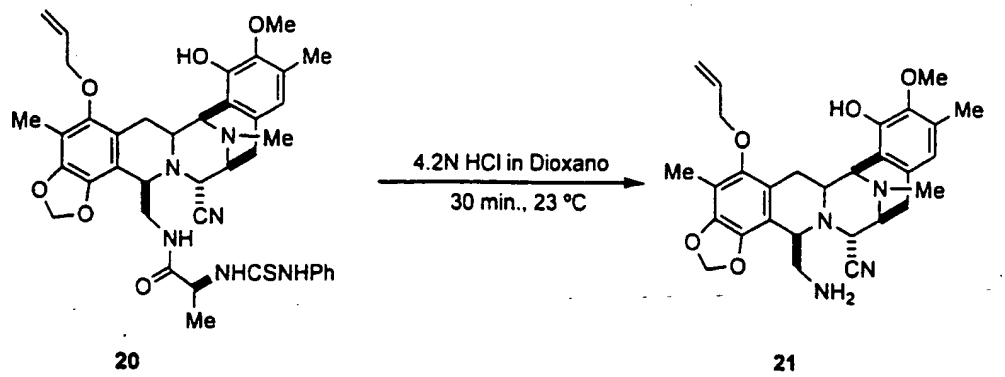
Rf: 0.56 ($CHCl_3$:ethyl acetate 1:4).

$^1$H NMR (300 MHz, $CDCl_3$): δ 8.00 (bs, 1H), 7.45-6.97 (m, 4H), 6.10 (s, 1H), 6.08-6.00 (m, 1H), 5.92 (s, 1H), 5.89 (s, 1H), 5.82 (s, 1H), 5.40 (dd, *J*= 1.5 Hz, *J*= 17.1 Hz, 1H), 3.38 (bs, 1H), 5.23 (dd, *J*= 1.5 Hz, *J*= 10.5 Hz, 1H), 4.42-4.36 (m, 1H), 4.19-4.03 (m, 5H), 3.71 (s, 3H), 3.68-3.17 (m, 4H), 2.90 (dd, *J*=7.8 Hz, *J*= 18.3 Hz, 1H), 2.57 (d, *J*= 18.3 Hz, 1H), 2.25 (s, 3H), 2.12 (s, 3H), 2.10 (s, 3H), 1.90 (dd, *J*= 12.3 Hz, *J*= 16.5 Hz, 1H), 0.81 (d, *J*= 6.9 Hz, 3H).

$^{13}$C NMR (75 MHz, $CDCl_3$): δ 178.4, 171.6, 148.6, 146.8, 144.3, 142.7, 138.7, 136.2, 133.6, 130.7, 129.8, 126.6, 124.2, 124.1, 120.9, 120.5, 117.7, 117.4, 116.7, 112.6, 112.5, 101.0, 74.0, 60.6, 59.0, 57.0, 56.2, 56.1, 55.0, 53.3, 41.4, 39.7, 26.3, 24.8, 18.3, 15.5, 9.2.

ESI-MS m/z: Calcd. for $C_{39}H_{44}N_6O_6S$: 724.8 Found $(M+H)^+$: 725.3.

Example 8




20 21

To a solution of **20** (270 mg, 0.37 ml) in dioxane (1 ml), 4.2N HCl/dioxane (3.5 ml) was added and the reaction was stirred at 23 °C for 30 min. Then, ethyl acetate (20 ml) and $H_2O$ (20 ml) were added and the organic layer was decanted. The aqueous phase was basified with saturated aqueous sodium bicarbonate (60 ml) (pH = 8) at 0 °C and then, extracted with $CH_2Cl_2$ (2 x 50 ml). The combined organic extracts were dried (sodium sulphate), and concentrated *in vacuo*. The residue was purified by flash column chromatography ($SiO_2$, ethyl acetate:methanol 5:1) to afford compound **21** (158 mg, 82%) as a white solid.

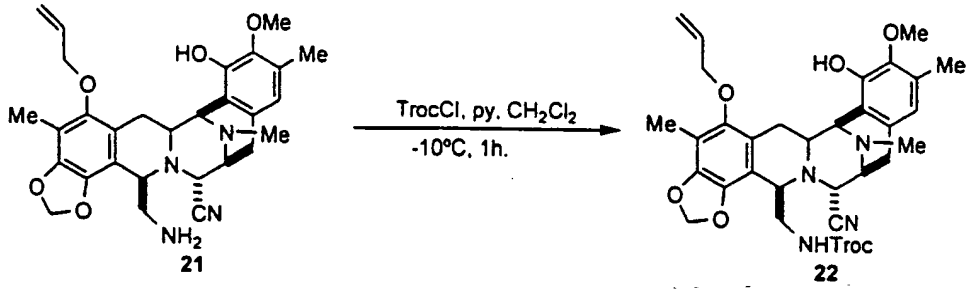
Rf: 0.3 (ethyl acetate:methanol 1:1).

$^1$H NMR (300 MHz, $CDCl_3$): δ 6.45 (s, 1H), 6.12-6.03 (m, 1H), 5.91 (s, 1H), 5.85 (s, 1H), 5.38 (dd, $J_1$= 1.2 Hz, $J_2$= 17.1 Hz, 1H), 5.24 (dd, $J_1$= 1.2 Hz, $J_2$= 10.5 Hz, 1H), 4.23-4.09 (m, 4H), 3.98 (d, $J$= 2.1 Hz, 1H), 3.90 (bs, 1H), 3.72 (s, 3H), 3.36-3.02 (m, 5H), 2.72-2.71 (m, 2H), 2.48 (d, $J$= 18.0 Hz, 1H), 2.33 (s, 3H), 2.22 (s, 3H), 2.11 (s, 3H), 1.85 (dd, $J_1$= 11.7 Hz, $J_2$= 15.6 Hz, 1H) ).

$^{13}$C NMR (75 MHz, $CDCl_3$): δ 148.4, 146.7, 144.4, 142.8, 138.8, 133.8, 130.5, 128.8, 121.5, 120.8, 118.0, 117.5, 116.9, 113.6, 112.2, 101.1, 74.3, 60.7, 59.9, 58.8, 56.6, 56.5, 55.3, 44.2, 41.8, 29.7, 26.5, 25.7, 15.7, 9.4.

ESI-MS m/z: Calcd. for $C_{29}H_{34}N_4O_5$: 518.3. Found $(M+H)^+$: 519.2.

Example 9




To a solution of **21** (0.64 g, 1.22 ml) in $CH_2Cl_2$ (6.13 ml), pyridine (0.104 ml, 1.28 ml) and 2,2,2-trichloroethyl chloroformate (0.177 ml, 1.28 ml) were added at -10 °C. The mixture was stirred at this temperature for 1h and then, the reaction was quenched by addition of 0.1N HCl (10 ml) and extracted with $CH_2Cl_2$ (2 x 10 ml). The organic layer was dried over sodium sulphate and concentrated *in vacuo*. The residue was purified by flash column chromatography ($SiO_2$, (hexane:ethyl acetate 1:2) to afford **22** (0.84 g, 98%) as a white foam solid.

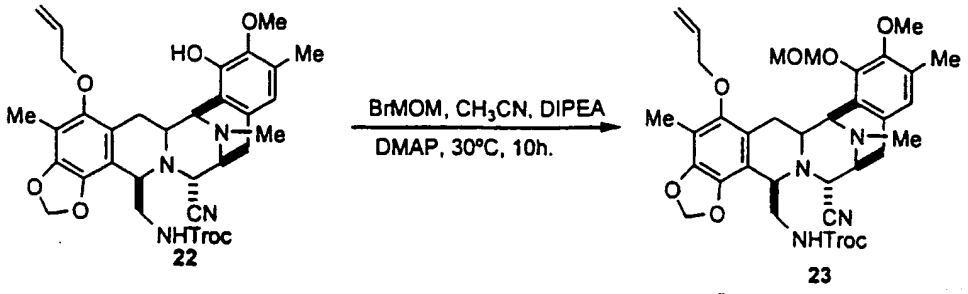
Rf: 0.57 (ethyl acetate:methanol 5:1).

$^1$H NMR (300 MHz, $CDCl_3$): δ 6.50 (s, 1H), 6.10-6.00 (m, 1H), 6.94 (d, $J$= 1.5 Hz, 1H), 5.87 (d, $J$= 1.5 Hz, 1H), 5.73 (bs, 1H), 5.37 (dq, $J_1$= 1.5 Hz, $J_2$= 17.1 Hz, 1H), 5.26 (dq, $J_1$= 1.8 Hz, $J_2$= 10.2 Hz, 1H), 4.60 (d, $J$= 12 Hz, 1H), 4.22-4.10 (m, 4H), 4.19 (d, $J$= 12 Hz, 1H), 4.02 (m, 2H), 3.75 (s, 3H), 3.37-3.18 (m, 5H), 3.04 (dd, $J_1$= 8.1 Hz, $J_2$= 18 Hz, 1H), 2.63 (d, $J$= 18 Hz, 1H), 2.31 (s, 3H), 2.26 (s, 3H), 2.11 (s, 3H), 1.85 (dd, $J_1$= 12.3 Hz, $J_2$= 15.9 Hz, 1H).

$^{13}$C NMR (75 MHz, $CDCl_3$) δ 154.3, 148.5, 146.7, 144.5, 142.8, 139.0, 133.8, 130.7, 128.7, 121.3, 120.8, 117.8, 117.7, 116.8, 112.7, 101.2, 77.2, 74.3, 60.7, 59.9, 57.0, 56.4, 55.3, 43.3, 41.7, 31.6, 26.4, 25.3, 22.6, 15.9, 14.1, 9.4.

ESI-MS m/z: Calcd. for $C_{32}H_{35}Cl_3N_4O_7$: 694.17. Found $(M+H)^+$: 695.2.

Example 10




To a solution of **22** (0.32 g, 0.46 ml) in $CH_3CN$ (2.33 ml), diisopropylethylamine (1.62 ml, 9.34 ml), bromomethyl methyl ether (0.57 ml, 7.0 ml) and dimethylaminopyridine (6 mg, 0.046 ml) were added at 0 °C. The mixture was heated at 30 °C for 10h. Then, the reaction was diluted with dichloromethane (30 ml) and poured in an aqueous solution of HCl at pH = 5 (10 ml). The organic layer was dried over sodium sulphate and the solvent was eliminated under reduced pressure to give a residue which was purified by flash column chromatography ($SiO_2$, hexane:ethyl acetate 2:1) to afford **23** (0.304 g, 88%) as a white foam solid.

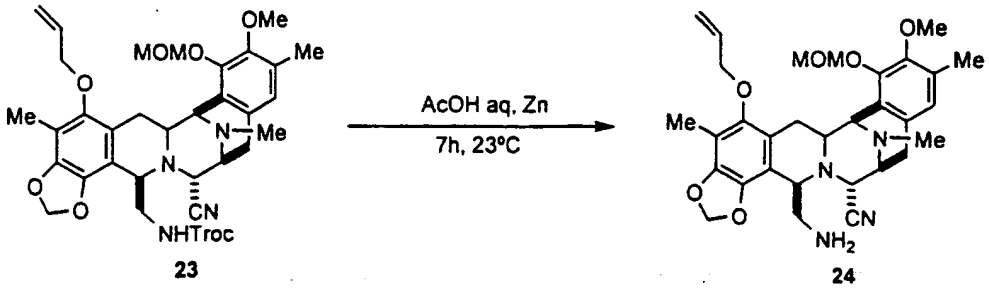
Rf: 0.62 (hexane:ethyl acetate 1:3).

$^1$H NMR (300 MHz, $CDCl_3$): δ 6.73 (s, 1H), 6.10 (m, 1H), 5.94 (d, $J$= 1.5 Hz, 1H), 5.88 (d, $J$= 1.5 Hz, 1H), 5.39 (dq, $J_1$= 1.5 Hz, $J_2$= 17.1 Hz, 1H), 5.26 (dq, $J_1$= 1.8 Hz, $J_2$= 10.2 Hz, 1H), 5.12 (s, 2H), 4.61 (d, $J$= 12 Hz, 1H), 4.55 (t, $J$= 6.6 Hz, 1H), 4.25 (d, $J$= 12 Hz, 1H), 4.22-4.11 (m, 4H), 4.03 (m, 2H), 3.72 (s, 3H), 3.58 (s, 3H), 3.38-3.21 (m, 5H), 3.05 (dd, $J_1$= 8.1 Hz, $J_2$= 18 Hz, 1H), 2.65 (d, $J$= 18 Hz, 1H), 2.32 (s, 3H), 2.23 (s, 3H), 2.12 (s, 3H), 1.79 (dd, $J_1$= 12.3 Hz, $J_2$= 15.9 Hz, 1H);

$^{13}$C NMR (75 MHz, $CDCl_3$) δ 154.3, 148.6, 148.4, 144.5, 139.0, 133.6, 130.6, 130.1, 125.07, 124.7, 124.0, 121.1, 117.7, 112.6, 101.2, 99.2, 77.2, 74.4, 74.1, 59.8, 59.8, 57.7, 57.0, 56.8, 56.68, 55.3, 43.2, 41.5, 26.4, 25.2, 15.9, 9.3.

ESI-MS m/z: Calcd. for $C_{34}H_{39}Cl_3N_4O_8$: 738.20. Found $(M+H)^+$: 739.0.

Example 11




23 24

To a suspension of **23** (0.304 g, 0.41 ml) in 90% aqueous acetic acid (4 ml), powder zinc (0.2 g, 6.17 ml) was added and the reaction was stirred for 7 hour at 23 °C. The mixture was filtered through a pad of celite which was washed with $CH_2Cl_2$. The organic layer was washed with an aqueous sat. solution of sodium bicarbonate (pH = 9) (15 ml) and dried over sodium sulphate. The solvent was eliminated under reduced pressure to give **24** (0.191 g, 83%) as a white solid.

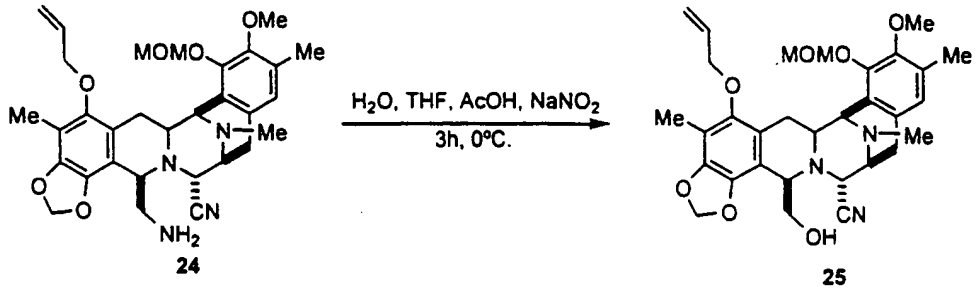
Rf: 0.3 (ethyl acetate:methanol 5:1).

$^1$H NMR (300 MHz, $CDCl_3$): δ 6.68 (s, 1H), 6.09 (m, 1H), 5.90 (d, $J$= 1.5 Hz, 1H), 5.83 (d, $J$= 1.5 Hz, 1H), 5.39 (dq, $J_1$= 1.5 Hz, $J_2$= 17.1 Hz, 1H), 5.25 (dq, $J_1$= 1.5 Hz, $J_2$= 10.2 Hz, 1H), 5.10 (s, 2H), 4.22-4.09 (m, 3H), 3.98 (d, $J$= 2.4 Hz, 1H), 3.89 (m, 1H), 3.69 (s, 3H), 3.57 (s, 3H), 3.37-3.17 (m, 3H), 3.07 (dd, $J_1$= 8.1 Hz, $J_2$= 18 Hz, 1H), 2.71 (m, 2H), 2.48 (d, $J$= 18 Hz, 1H), 2.33 (s, 3H), 2.19 (s, 3H), 2.17 (s, 3H), 1.80 (dd, $J_1$= 12.3 Hz, $J_2$= 15.9 Hz, 1H)

$^{13}$C NMR (75 MHz, $CDCl_3$): δ 148.5, 148.2, 144.3, 138.7, 133.7, 130.7, 129.9, 125.0, 123.9, 121.3, 117.9, 117.5, 113.6, 112.0, 101.0, 99.2, 74.0, 59.8, 59.7, 58.8, 57.6, 57.0, 56.2, 55.2, 44.2, 41.5, 31.5, 26.4, 25.6, 22.5, 16.7, 14.0, 9.2.

ESI-MS m/z: Calcd. for $C_{31}H_{38}N_4O_6$: 562.66. Found $(M+H)^+$: 563.1.

Example 12




To a solution of **24** (20 mg, 0.035 ml), in $H_2O$ (0.7 ml) and THF (0.7 ml), $NaNO_2$ (12 mg, 0.17 ml) and 90% aqueous AcOH (0.06 ml) were added at 0 °C and the mixture was stirred at 0 °C for 3h. After dilution with $CH_2Cl_2$ (5 ml), the organic layer was washed with water (1 ml), dried over sodium sulphate and concentrated *in vacuo*. The residue was purified by flash column chromatography ($SiO_2$, hexane:ethyl acetate 2:1) to afford **25** (9.8 mg, 50%) as a white solid.

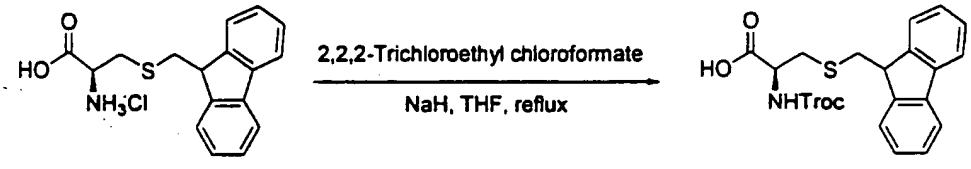
Rf: 0.34 (hexane:ethyl acetate 1:1).

$^1$H NMR (300 MHz, $CDCl_3$): δ 6.71 (s, 1H), 6.11 (m, 1H), 5.92 (d, $J$= 1.5 Hz, 1H), 5.87 (d, $J$= 1.5 Hz, 1H), 5.42 (dq, $J_1$= 1.5 Hz, $J_2$= 17.1 Hz, 1H), 5.28 (dq, $J_1$= 1.5 Hz, $J_2$= 10.2 Hz, 1H), 5.12 (s, 2H), 4.26-4.09 (m, 3H), 4.05 (d, $J$= 2.4 Hz, 1H), 3.97 (t, $J$= 3.0 Hz, 1H), 3.70 (s, 3H), 3.67-3.32 (m, 4H), 3.58 (s, 3H), 3.24 (dd, $J_1$= 2.7 Hz, $J_2$= 15.9 Hz, 1H), 3.12 (dd, $J_1$= 8.1 Hz, $J_2$= 18.0 Hz, 1H), 2.51 (d, $J$= 18 Hz, 1H), 2.36 (s, 3H), 2.21 (s, 3H), 2.12 (s, 3H), 1.83 (dd, $J_1$= 12.3 Hz, $J_2$= 15.9 Hz, 1H)

$^{13}$C NMR (75 MHz, $CDCl_3$) δ 148.7, 148.4, 138.9, 133.7, 131.1, 129.4, 125.1, 123.9, 120.7, 117.6, 117.5, 113.2, 112.3, 101.1, 99.2, 74.0, 63.2, 59.8, 59.7, 57.9, 57.7, 57.0, 56.5, 55.2, 41.6, 29.6, 26.1, 25.6, 22.6, 15.7, 9.2.

ESI-MS m/z: Calcd. for $C_{31}H_{37}N_3O_7$: 563.64. Found $(M+H)^+$: 564.1.

Example 13




**29**

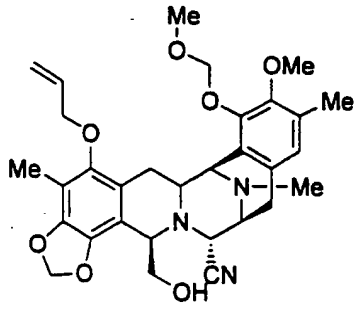
The starting material (2.0 g, 5.90 ml) was added to a suspension of sodium hydride (354 mg, 8.86 ml) in THF (40 ml) at 23 °C, following the suspension was treated with allyl chloroformate (1.135 ml, 8.25 ml) at 23 °C and then refluxed for 3 hours. The suspension was cooled, filtered off, the solid washed with ethyl acetate (100 ml), and the filtrate was concentrated. The oil crude was ground with hexane (100 ml) and kept at 4°C overnight. After, the solvent was decanted and the light yellow slurry was treated with $CH_2Cl_2$ (20 ml), and precipitated with hexane (100 ml). After 10 minutes, the solvent was decanted again. The operation was repeated until appearing a white solid. The white solid was filtered off and dried to afford compound **29** (1.80 g, 65%) as a white solid.

$^1$H-NMR (300 MHz, $CDCl_3$): δ 7.74 (d, *J*= 7.5 Hz, 2H), 7.62 (d, *J*= 6.9 Hz, 2H), 7.33 (t, *J*= 7.5 Hz, 2H), 7.30 (t, *J*= 6.3 Hz, 2H), 5.71 (d, *J*= 7.8 Hz, 1H), 4.73 (d, *J*= 7.8 Hz, 2H), 4.59 (m, 1H), 4.11 (t, *J*= 6.0 Hz, 1H), 3.17 (dd, *J*= 6.0 Hz, *J*= 2.7 Hz, 2H), 3.20 (dd, *J*= 5.4 Hz, *J*= 2.1 Hz, 2H).

$^{13}$C-NMR (75 MHz, $CDCl_3$): δ 173.6, 152.7, 144.0, 139.7, 137.8, 126.0, 125.6, 123.4, 118.3, 73.4, 52.4, 45.5, 35.8, 33.7.

ESI-MS m/z: Calcd.. for $C_{20}H_{18}Cl_3NO_4S$: 474.8. Found $(M+Na)^+$: 497.8

Example 14




A mixture of compound **25** (585 mg, 1.03 ml) and compound **29** (1.47 mg, 3.11 ml) were azeotroped with anhydrous toluene (3 x 10 ml). To a solution of **25** and **29** in anhydrous $CH_2Cl_2$ (40 ml) was added DMAP (633 mg, 5.18 ml) and EDC·HCl (994 mg, 5.18 ml) at 23

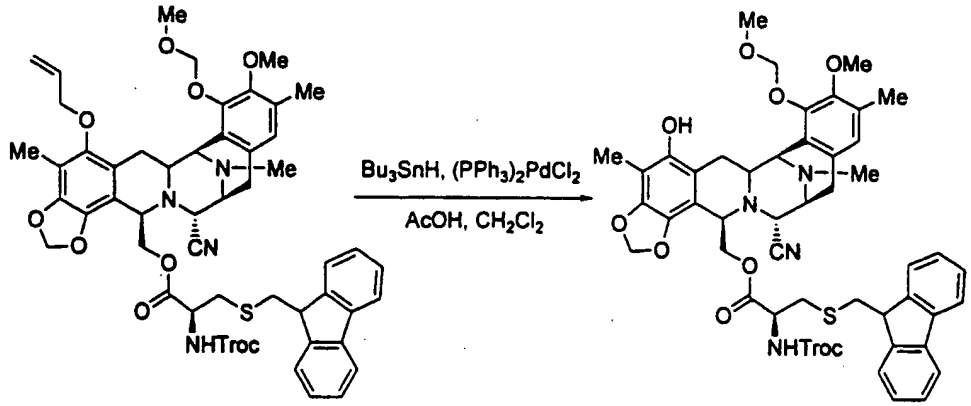
°C. The reaction mixture was stirred at 23 °C for 3 hours. The mixture was partitioned with saturated aqueous solution of sodium bicarbonate (50 ml ) and the layers were separated. The aqueous layer was washed with $CH_2Cl_2$ (50 ml). The combined organic layers were dried over sodium sulphate, filtered and concentrated. The crude was purified by flash column chromatography (ethyl acetate/hexane 1:3) to obtain **30** (1.00 g, 95%) as a pale cream yellow solid.

$^1$H-NMR (300 MHz, $CDCl_3$): δ 7.72 (m, 2H), 7.52 (m, 2H), 7.38 (m, 2H), 7.28 (m, 2H), 6.65 (s, 1H), 6.03 (m, 1H), 5.92 (d, *J*= 1.5 Hz, 1H), 5.79 (d, *J*= 1.5 Hz, 1H), 5.39 (m, 1H), 5.29 (dq, *J*= 10.3 Hz, *J*= 1.5 Hz, 1H), 5.10 (s, 2H), 4.73 (d, *J*= 11.9 Hz, 1H), 4.66 (d, *J*= 11.9 Hz, 1H), 4.53 (m, 1H), 4.36-3.96 (m, 9H), 3.89 (t, *J*= 6.4 Hz, 1H), 3.71 (s, 3H), 3.55 (s, 3H), 3.33 (m, 1H), 3.20 (m, 2H), 2.94 (m, 3H), 2.59 (m, 1H), 2.29 (s, 3H), 2.23 (s, 3H), 2.02 (s, 3H), 1.83 (dd, *J*= 16.0 Hz, *J*= 11.9 Hz, 1H).

$^{13}$C-NMR (75 MHz, $CDCl_3$): δ 169.7, 154.0, 148.8, 148.4, 145.7, 144.5, 140.9, 139.0, 133.7, 130.9, 130.6, 127.6, 127.0, 124.8, 124.6, 124.1, 120.8, 119.9, 118.2, 117.7, 117.3, 112.7, 112.1, 101.3, 99.2, 74.7, 73.9, 64.4, 59.8, 57.7, 57.0, 56.8, 55.4, 53.3, 46.7, 41.4, 36.5, 34.7, 31.5, 26.4, 24.9, 22.6, 15.7, 14.0, 9.1.

ESI-MS m/z: Calcd.. for $C_{51}H_{53}Cl_3N_4O_{10}S$: 1020.4. Found $(M+H)^+$: 1021.2

Example 15




**30** **31**

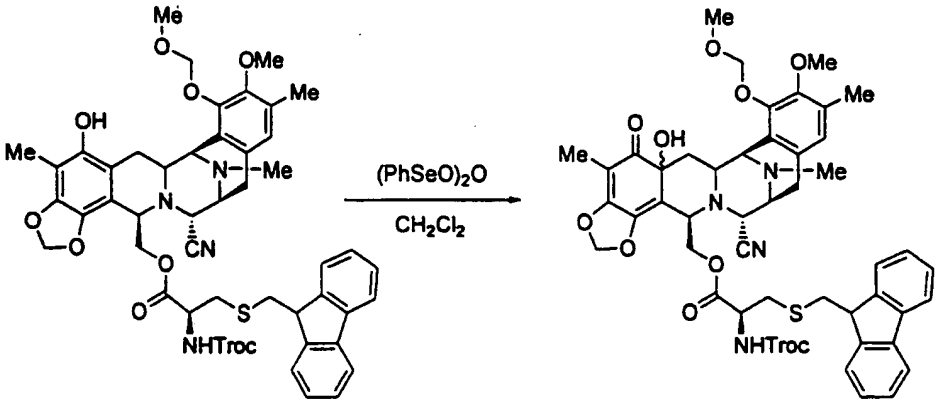
To a solution of **30** (845 mg, 0.82 ml), acetic acid (500 mg, 8.28 ml) and $(PPh_3)_2PdCl_2$ (29 mg, 0.04 ml) in anhydrous $CH_2Cl_2$ 20 ml at 23 °C was added, dropwise, $Bu_3SnH$ (650 mg, 2.23 ml). The reaction mixture was stirred at this temperature for 15 min., bubbling was. The crude was quenched with water (50ml) and extracted with $CH_2Cl_2$ (3 x 50 ml). The organic layers were dried over sodium sulphate, filtered and concentrated. The crude was purified by flash column chromatography (ethyl acetate/hexane in gradient from 1:5 to 1:3) to obtain compound **31** (730 mg, 90%) as a pale cream yellow solid.

$^1$H-NMR (300 MHz, $CDCl_3$): δ 7.72 (m, 2H), 7.56 (m, 2H), 7.37 (m, 2H), 7.30 (m, 2H), 6.65 (s, 1H), 5.89 (s, 1H), 5.77 (s, 1H), 5.74 (s, 1H), 5.36 (d, *J*= 5.9 Hz, 1H), 5.32 (d, *J*= 5.9 Hz, 1H), 5.20 (d, *J*= 9.0, 1H), 4.75 (d, *J*= 12.0 Hz, 1H), 4.73 (m, 1H), 4.48 (d, *J*= 11.9 Hz, 1H), 4.08 (m, 4H), 3.89 (m, 1H), 3.86, (t, *J*= 6.2 Hz, 1H), 3.70 (s, 3H), 3.69 (s, 3H), 3.38 (m, 1H), 3.25 (m, 1H), 3.02-2.89 (m, 4H), 2.67 (s, 1H), 2.61 (s, 1H ), 2.51 (dd, *J*= 14.3 Hz, *J*= 4.5 Hz, 1H), 2.29 (s, 3H), 2.23 (s, 3H), 1.95 (s, 3H), 1.83 (m, 1H).

$^{13}$C-NMR (75 MHz, $CDCl_3$): δ 168.2, 152.5, 148.1, 146.2, 144.4, 144.3, 143.3, 139.6, 134.6, 129.7, 129.6, 126.2, 125.6, 123.4, 123.3, 121.6, 118.5, 116.3, 110.7, 110.2, 105.1, 99.4, 98.5, 75.2, 73.3, 61.7, 58.4, 57.9, 56.3, 56.1, 55.1, 54.7, 53.9, 51.9, 45.2, 40.1, 35.6, 33.3, 24.8, 23.3., 14.5, 7.3.

ESI-MS m/z: Calcd.. for $C_{48}H_{49}Cl_3N_4O_{10}S$: 980.3. Found $(M+H)^+$: 981.2

Example 16

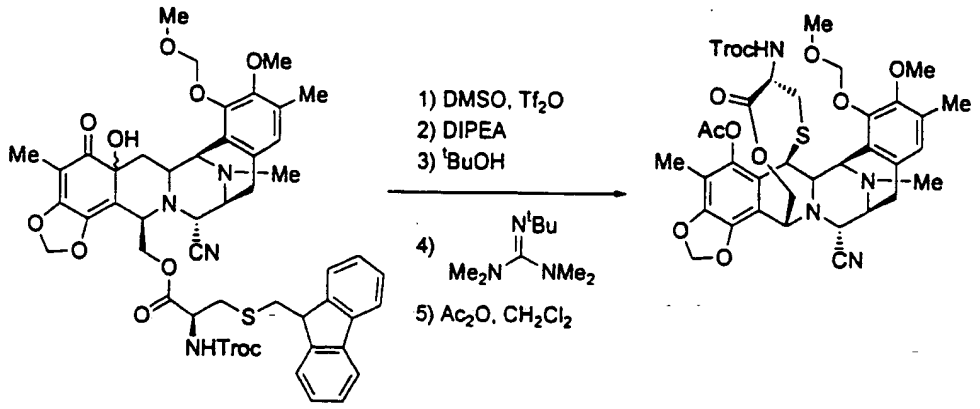



**31** **32**

To a solution of **31** (310 mg, 0.32 ml), in anhydrous $CH_2Cl_2$ (15 ml) at -10 °C was added a solution of benzeneseleninic anhydride 70 % (165 mg, 0.32 ml), in anhydrous $CH_2Cl_2$ (7 ml), *via* cannula, keeping the temperature at -10 °C. The reaction mixture was stirred at -10 °C for 5 min. A saturated solution of sodium bicarbonate (30 ml) was added at this temperature. The aqueous layer was washed with more $CH_2Cl_2$ (40 ml). The organic layers were dried over sodium sulphate, filtered and concentrated. The crude was purified by flash column chromatography (ethyl acetate/hexane in gradient from 1:5 to 1:1) to obtain 32 (287 mg, 91%, HPLC: 91.3%) as a pale cream yellow solid and as a mixture of two isomers (65:35) which were used in the next step.

$^1$H-NMR (300 MHz, $CDCl_3$): δ (Mixture of isomers) 7.76 (m, 4H), 7.65 (m, 4H), 7.39 (m, 4H), 7.29 (m, 4H), 6.62 (s, 1H), 6.55 (s, 1H), 5.79-5.63 (m, 6H), 5.09 (s, 1H), 5.02 (d, *J*= 6.0 Hz, 1H), 4.99 (d, *J*= 6.0 Hz, 1H), 4.80-4.63 (m, 6H), 4.60 (m, 1H), 4.50 (m, 1H), 4.38 (d, *J*= 12.8 Hz, *J*= 7.5 Hz, 1H), 4.27 (dd, *J*= 12.8 Hz, *J*= 7.5 Hz, 1H), 4.16-3.90 (m, 10H), 3.84 (s, 3H), 3.62 (s, 3H), 3.50 (s, 3H), 3.49 (s, 3H), 3.33-2.83 (m, 14H), 2.45-2.18 (m, 2H), 2.21 (s, 6H), 2.17 (s, 6H ), 1.77 (s, 6H), 1.67 (m, 2H).
$^{13}$C-NMR (75 MHz, $CDCl_3$): δ (Mixture of isomers) 168.6, 168.4, 158.6, 154.8, 152.8, 152.5, 147.3, 147.2, 146.8, 144.1, 144.0, 140.8, 139.7, 137.1, 129.8, 129.3, 128.4, 128.7, 126.5, 125.5, 123.7, 123.6, 123.5, 123.4, 122.2, 121.3, 118.3, 115.8, 115.5, 110.2, 106.9, 103.5, 103.2, 100.1, 99.6, 97.9, 97.7, 93.8, 73.4, 70.9, 69.2, 64.9, 62.5, 59.3, 58.9, 58.4, 56.7, 56.3, 56.2, 55.4, 55.2, 55.1, 54.9, 54.7, 54.3, 54.1, 53.8, 52.8, 45.5, 40.5, 40.0, 39.8, 35.8, 35.5, 33.9, 33.7, 30.1, 28.8, 24.2, 24.1, 21.2, 14.5, 14.4, 12.7, 6.0, 5.7.
ESI-MS m/z: Calcd.. for $C_{48}H_{49}Cl_3N_4O_{11}S$: 996.3. Found $(M+H)^+$: 997.2

Example 17




32 33

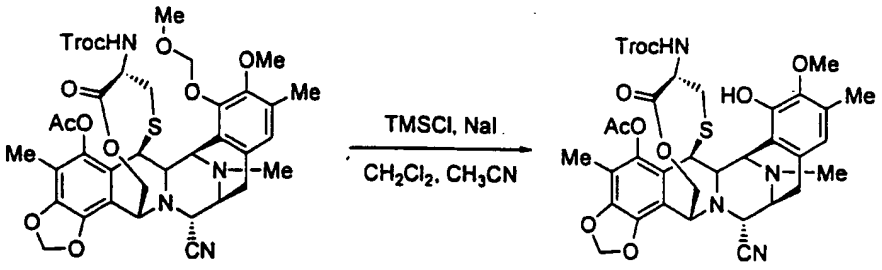
The reaction flask was flamed twice, purged vacuum/Argon several times and kept under Argon atmosphere for the reaction. To a solution of DMSO (39.1 ml, 0.55 ml, 5 equivalents.) in anhydrous $CH_2Cl_2$ (4.5 ml) was dropwise added triflic anhydride (37.3 ml, 0.22 ml, 2 equivalents.) at -78 °C . The reaction mixture was stirred at -78 °C for 20 minutes, then a solution of **32** (110 mg, 0.11 ml, HPLC: 91.3%) in anhydrous $CH_2Cl_2$ (1 ml, for the main addition and 0.5 ml for wash) at -78 °C was added, *via* cannula. During the addition the temperature was kept at -78 °C in both flasks and the colour changed from yellow to brown. The reaction mixture was stirred at -40 °C for 35 minutes. During this period of time the solution was turned from yellow to dark green. After this time, $^iPr_2NEt$ (153 ml, 0.88 ml, 8 equivalents.) was dropwise added and the reaction mixture was kept at 0 °C for 45 minutes, the colour of the solution turned to brown during this time. Then t-butanol (41.6 ml, 0.44 ml, 4 equivalents.) and 2-$^t$Butyl-1,1,3,3-tetramethylguanidine (132.8 ml, 0.77 ml, 7 equivalents.) were dropwise added and the reaction mixture was stirred at 23 °C for 40 minutes. After this time, acetic anhydride (104.3 ml, 1.10 ml, 10 equivalents.) was dropwise added and the reaction mixture was kept at 23 °C for 1 hour more. Then the reaction mixture was diluted with $CH_2Cl_2$ (20ml) and washed with aqueous saturated solution of $NH_4Cl$ (50ml), sodium bicarbonate (50ml), and sodium chloride (50ml). The combined organic layers were dried over sodium sulphate, filtered and concentrated. The residue was purified by flash column chromatography (eluent: ethyl acetate/hexane gradient from 1:3 to 1:2) to afford compound **33** (54 mg, 58%) as a pale yellow solid.

$^1$H-NMR (300 MHz, $CDCl_3$): δ 6.85 (s, 1H), 6.09 (s, 1H), 5.99 (s, 1H), 5.20 (d, *J*= 5.8 Hz, 1H), 5.14 (d, *J*= 5.3 Hz, 1H), 5.03 (m, 1H), 4.82 (d, *J*= 12.2, 1H), 4.63 (d, *J*= 12.0 Hz, 1H), 4.52 (m, 1H), 4.35-4.17 (m, 4H), 3.76 (s, 3H), 3.56 (s, 3H), 3.45 (m, 2H), 2.91 (m, 2H), 2.32 (s, 3H), 2.28 (s, 3H), 2.21 (s, 3H), 2.12 (m, 2H), 2.03 (s, 3H).

$^{13}$C-NMR (75 MHz, $CDCl_3$): δ 168.5, 167.2, 152.7, 148.1, 147.1, 144.5, 139.6, 139.1, 130.5, 129.0, 123.7, 123.5, 123.3, 118.8, 116.5, 112.1, 100.6, 97.8, 73.3, 60.5, 59.4, 59.2, 58.3, 57.6, 57.4, 56.1, 53.3, 53.1, 40.6, 40.0, 31.0, 22.2, 18.9, 14.4, 8.1.

ESI-MS m/z: Calcd.. for $C_{36}H_{39}Cl_3N_4O_{11}S$: 842.1. Found $(M+H)^+$: 843.1

Example 18




**33** **34**

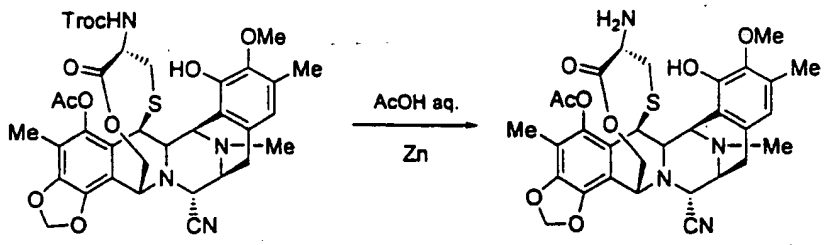
To a solution of **33** (12 mg, 0.014 ml)in dry dichloromethane (1.2 ml) and HPLC grade acetonitrile (1.2 ml) was added at 23 °C sodium iodide (21 mg, 0.14 ml) and freshly distilled (over calcium hydride at atmospheric pressure) trimethylsilyl chloride (15.4 mg, 0.14 ml). The reaction mixture turned to orange colour. After 15 min the solution was diluted with dichloromethane (10 ml) and was washed with a freshly aqueous saturated solution of $Na_2S_2O_4$ (3 x 10 ml). The organic layer was dried over sodium sulphate, filtered and concentrated. It was obtained compound **34** (13 mg, quantitative) as pale yellow solid which was used without further purification.

$^1$H-NMR (300 MHz, $CDCl_3$): δ 6.85 (s, 1H), 6.09 (s, 1H), 5.99 (s, 1H), 5.27 (d, *J*= 5.8 Hz, 1H), 5.14 (d, *J*= 5.3 Hz, 1H), 5.03 (d, *J*= 11.9 Hz, 1H), 4.82 (d, *J*= 12.2, 1H), 4.63 (d, *J*= 13.0 Hz, 1H), 4.52 (m, 1H), 4.34 (m, 1H), 4.27 (bs, 1H), 4.18 (m, 2H), 3.76 (s, 3H), 3.56 (s, 3H), 3.44 (m, 1H), 3.42 (m, 1H), 2.91 (m, 2H), 2.32 (s, 3H), 2.28 (s, 3H), 2.21 (s, 3H), 2.03 (s,

3H).

ESI-MS m/z: Calcd.. for $C_{34}H_{35}N_4O_{10}S$: 798.1. Found $(M+H)^+$: 799.1

Example 19

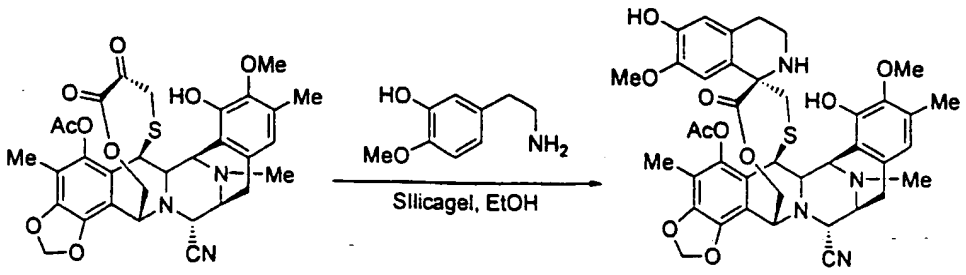



34 35

To a solution of **34** (13 mg, 0.016 ml) in a mixture of acetic acid/$H_2O$ (90:10, 1 ml) was added powder Zinc (5.3 mg, 0.081 ml) at 23 °C. The reaction mixture was heated at 70 °C for 6 h. After this time, was cooled to 23 °C, diluted with $CH_2Cl_2$ (20 ml) and washed with aqueous saturated solution of sodium bicarbonate (15 ml) and aqueous solution of $Et_3N$ (15 ml). The organic layer was dried over sodium sulphate, filtered and concentrated. The residue was purified by flash column chromatography with Silica-$NH_2$ (eluent: ethyl acetate/hexane gradient from 0:100 to 50:50) to afford compound **35** (6.8 mg, 77% for two steps) as a pale yellow solid.

$^1$H-NMR (300 MHz, $CDCl_3$): δ 6.51 (s, 1H), 6.03 (dd, *J*= 1.3 Hz, *J*= 26.5 Hz, 2H), 5.75 (bs, 1H), 5.02 (d, *J*= 11.6 Hz, 1H), 4.52 (m, 1H), 4.25 (m, 2H), 4.18 (d, *J*= 2.5 Hz, 1H), 4.12 (dd, *J*= 1.9 Hz, *J*= 11.5 Hz, 1H), 3.77 (s, 3H), 3.40 (m, 2H), 3.26 (t, *J*= 6.4 Hz, 1H), 2. 88 (m, 2H), 2.30-2.10 (m, 2H), 2.30 (s, 3H), 2.28 (s, 3H), 2.18 (s, 3H), 2.02 (s, 3H).
$^{13}$C-NMR (75 MHz, $CDCl_3$): δ 174.1, 168.4, 147.8, 145.4, 142.9, 140.8, 140.1, 131.7, 130.2, 129.1, 128.3, 120.4, 118.3, 117.9, 113.8, 111.7, 101.7, 61.2, 59.8, 59.2, 58.9, 54.4, 53.8, 54.4, 41.3, 41.5, 34.1, 23.6, 20.3, 15.5, 9.4.
ESI-MS m/z: Calcd.. for $C_{31}H_{34}N_4O_8S$: 622.7. Found $(M+H)^+$: 623.2.

Example 20



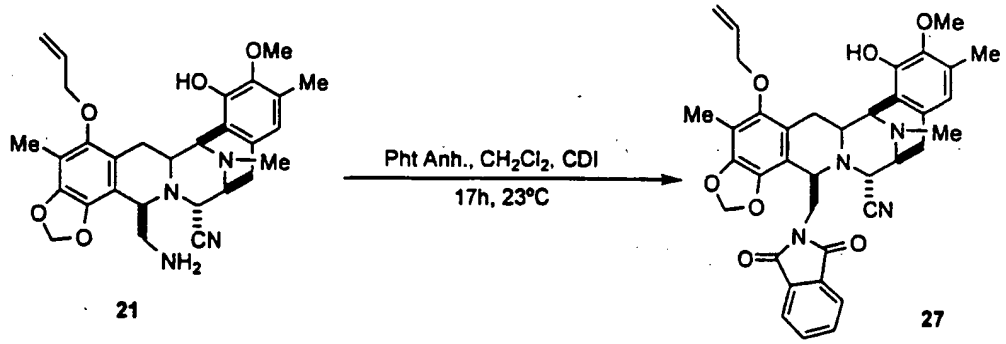

36 Et-770

To a solution of **36** (49mg, 0.08 ml) and 2-[3-hydroxy-4-methoxyphenyl]ethylamine (46.2 mg, 0.27 ml) in ethanol (2.5 ml) was added silica gel (105 mg) at 23 °C. The reaction mixture was stirred at 23 °C for 14 h. It was diluted with hexane and poured into a column of chromatography (ethyl acetate/hexane from 1/3 to 1/1) to afford **Et-770** (55 mg, 90%) as a pale yellow solid.

$^1$H-NMR (300 MHz, $CDCl_3$): δ 6.60 (s, 1H), 6.47 (s, 1H), 6.45 (s, 1H), 6.05 (s, 1H), 5.98 (s, 1H), 5.02 (d, *J*=11.4 Hz, 1H), 4.57 (bs, 1H), 4.32 (bs, 1H), 4.28 (d, *J*= 5.3 Hz, 1H), 4.18 (d, *J*= 2.5 Hz, 1H), 4.12 (dd, *J*= 2.1 Hz, *J*= 11.5 Hz, 1H), 3.78 (s, 3H), 3.62 (s, 3H), 3.50 (d, *J*= 5.0 Hz, 1H), 3.42 (m, 1H), 3.10 (ddd, *J*= 4.0 Hz, *J*= 10.0 Hz, *J*= 11.0 Hz, 1H), 2.94 (m, 2H), 2.79 (m, 1H), 2.61 (m, 1H), 2.47 (m, 1H), 2.35 (m, 1H), 2.32 (s, 3H), 2.27 (s, 3H), 2.20 (s, 3H), 2.09 (m, 1H), 2.04 (s, 3H).

ESI-MS m/z: Calcd.. for $C_{40}H_{42}N_4O_{10}S$: 770.7. Found $(M+H)^+$: 771.2

Example 22




21 27

To a solution of **21** (22 mg, 0.042 ml) in $CH_2Cl_2$ (0.8 ml) was added phthalic anhydride (6.44 mg, 0.042 ml) and the reaction mixture was stirred for 2h at 23 °C. Then, carbonyldiimidazole (1mg, 0.006 ml) was added and the mixture was stirred at 23 °C for 7h. Then, carbonyldiimidazole (5.86mg, 0.035 ml) was added and the reaction was stirred at 23 °C for an additional 17h. The solution was diluted with $CH_2Cl_2$ (15 ml) and washed with 0.1 N HCl (15 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography ($SiO_2$, hexane:ethyl acetate 2:1) to afford **27** (26.4 mg, 96%) as a white solid.

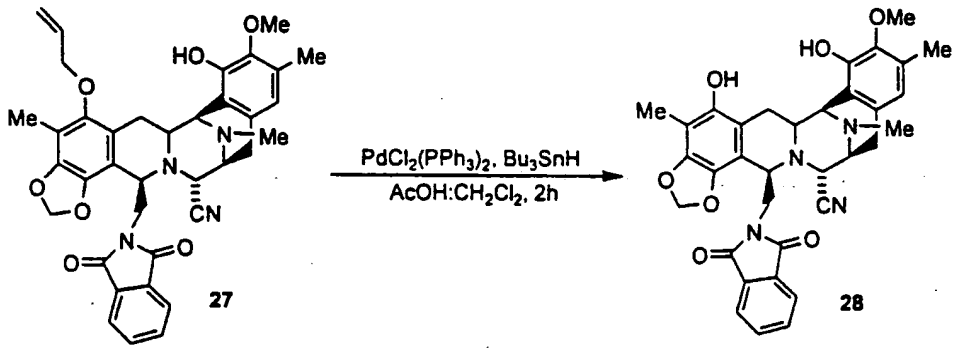
Rf: 0.58 (ethyl acetate).

$^1$H NMR (300 MHz, $CDCl_3$): 7.73–7.64 (m, 4H), 6.40 (s, 1H), 6.12-6.01 (m, 1H), 5.63 (s, 1H), 5.58 (d, $J$= 1.5 Hz, 1H), 5.37 (dd, $J_1$= 1.8 Hz, $J_2$= 17.4 Hz), 5.23 (dd, $J_1$= 1.8 Hz, $J_2$= 10.5 Hz, 1H), 5.12 (d, $J$= 1.5 Hz, 1H), 4.22-4.15 (m, 3H), 4.08 (d, $J$= 1.8 Hz, 1H), 3.68 (s, 3H), 3.59-3.55 (m 2H), 3.35 (d, $J$= 8.1 Hz, 1H), 3.27-3.16 (m, 2H), 3.05 (dd, $J_1$= 8.1 Hz, $J_2$= 18.3 Hz, 1H), 2.64 (d, $J$= 18.0Hz, 1H), 2.30 (s, 3H), 2.24 (s, 3H), 2.09 (s, 3H), 1.80 (dd, $J_1$= 11.4 Hz, $J_2$= 15 Hz, 1H);

$^{13}$C NMR (75 MHz, $CDCl_3$): δ 167.7, 148.9, 146.4, 144.2, 142.6, 139.5, 134.0, 133.5, 132.0, 131.0, 128.3, 123.0, 121.3, 120.9, 118.1, 117.5, 116.8, 113.6, 112.4, 100.8, 74.5, 60.6, 60.5, 57.7, 56.6, 55.6, 55.5, 42.3, 41.7, 26.6, 25.5, 15.9, 9.46.

ESI-MS m/z: Calcd. for $C_{37}H_{35}N_4O_7$: 648.79. Found $(M+H)^+$: 649.3.

Example 23



To a solution of **27** (26 mg, 0.041 ml) in $CH_2Cl_2$ (11 ml), acetic acid (11 ml), $(PPh_3)_2PdCl_2$ (2.36 mg) and $Bu_3SnH$ (28 ml, 0.10 ml) were added at 23 °C. After stirring at that temperature for 2h the reaction was poured into a pad of flash column ($SiO_2$, gradient Hex to hexane:ethyl acetate 2:1) to afford **28** (24.7 mg, 99 %) as a white solid.

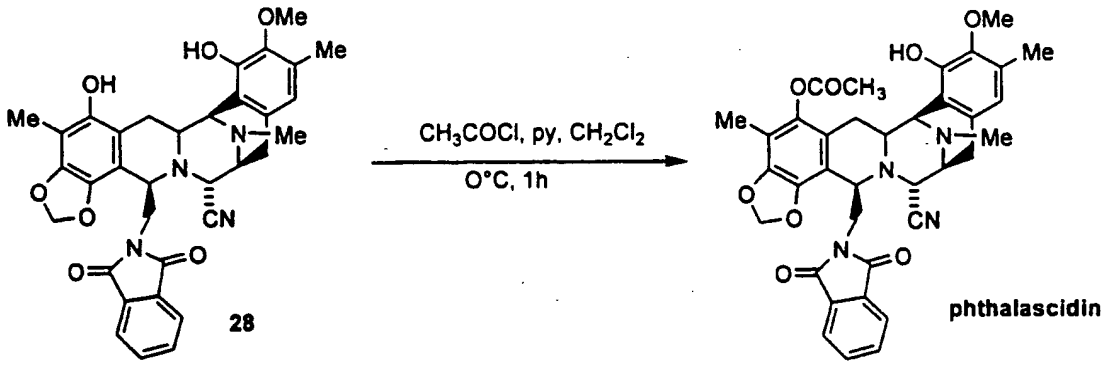
Rf: 0.33 (hexane:ethyl acetate 2:1).

$^1H$ NMR (300 MHz, $CDCl_3$): δ 7.75-7.70 (m, 2H), 7.69-7.65 (m, 2H), 6.39 (s, 1H), 5.82 (bs, 1H), 5.50 (d, *J*= 1.5 Hz, 1H), 5.0 (d, *J*= 1.5 Hz, 1H), 4.45 (bs, 1H), 4.23-4.19 (m, 2H), 4.10-4.09 (m, 1H), 3.73 (s, 3H), 3.60-3.48 (m, 2H), 3.36-3.33 (m, 1H), 3.26-3.20 (m, 1H), 3.14-3.08 (m, 1H), 3.98 (d, *J*= 14.4 Hz, 1H), 2.61 (d, *J*= 18.3 Hz, 1H), 2.30 (s, 3H), 2.23 (s, 3H), 2.06 (s, 3H), 1.85 (dd, $J_1$= 12 Hz, $J_2$= 15.3 Hz);

$^{13}C$ NMR (75 MHz, $CDCl_3$): δ 167.8, 146.4, 145.1, 143.9, 142.7, 137.1, 133.5, 131.9, 130.8, 128.4, 122.9, 120.8, 118.0, 116.8, 114.0, 113.4, 106.4, 100.4, 60.6, 60.5, 57.8, 56.6, 55.5, 55.2, 42.6, 41.5, 25.6, 25.5, 15.8, 8.9.

ESI-MS m/z: Calcd. for $C_{34}H_{32}N_4O_7$: 608.6. Found $(M+H)^+$: 609.2.

Example 24




To a solution of **28** (357 mg, 0.058 ml) in $CH_2Cl_2$ (3 ml), acetyl chloride (41.58 ml, 0.58 ml) and pyridine (47.3 ml, 0.58 ml) were added at 0 °C. The reaction mixture was stirred for 1h and then, the solution was diluted with $CH_2Cl_2$ (15 ml) and washed with 0.1 N HCl (15 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column

chromatography (RP-18, $CH_3CN:H_2O$ 60:40) to afford phthalascidin (354 mg, 94%) as a white solid.

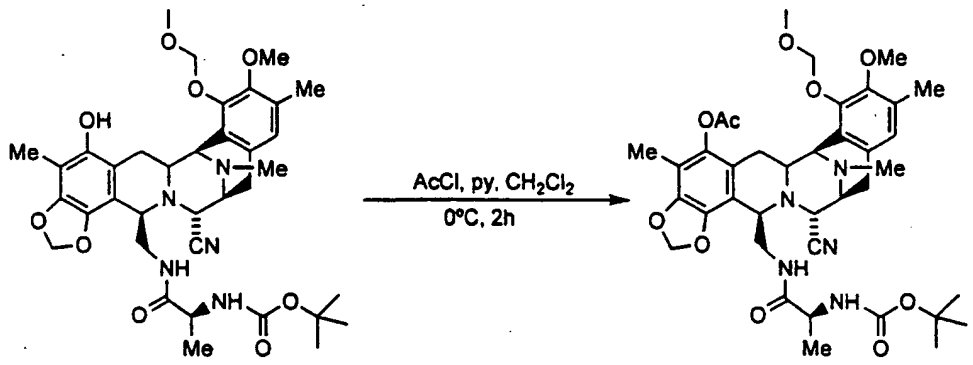
Rf: 0.37 ($CH_3CN:H_2O$ 7:3, RP-18).

$^1H$ NMR (300 MHz, $CDCl_3$): δ 7.72–7.68 (m, 2H), 7.67-7.63 (m, 2H), 6.38 (s, 1H), 5.69 (d, $J$= 1.2 Hz, 1H), 5.64 (d, $J$= 1.2Hz, 1H), 5.30 (bs, 1H), 4.25-4.21 (m, 2H), 4.02 (d, $J$= 2.1 Hz, 1H), 3.64-3.62 (m, 5H), 3.33 (d, $J$= 8.4 Hz, 1H), 3.21-3.16 (m, 1H), 3.02 (dd, $J_1$= 8.1 Hz, $J_2$= 18 Hz, 1H), 2.76 (dd, $J_1$= 1.8 Hz, $J_2$= 15.6 Hz, 1H), 2.63 (d, $J$= 17.7 Hz, 1H), 2.29 (s, 3H), 2.28 (s,3H), 2.21 (s, 3H), 2.0 (s, 3H), 1.73 (dd, $J_1$= 12.0 Hz, $J_2$= 15.3 Hz, 1H) )

$^{13}C$ NMR (75 MHz, $CDCl_3$) ): δ 168.5, 167.6, 146.2, 144.2, 142.5, 141.0, 140.5, 133.4, 131.8, 130.7, 128.2, 120.9, 120.8, 117.9, 116.4, 113.6, 101.1, 60.4, 60.0, 57.0, 56.3, 55.6, 55.4, 41.6, 41.5, 26.5, 25.2, 20.2, 15.7, 9.4.

ESI-MS m/z: Calcd. for $C_{36}H_{34}N_4O_8$: 650. Found $(M+H)^+$: 651.2.

Example 25




17 42

To a solution of **17** (300 mg, 0.432 ml) in $CH_2Cl_2$ (2 ml), acetyl chloride (30.7 ml, 0.432 ml) and pyridine (34.9 ml, 0.432 ml) were added at 0 °C. The reaction mixture was stirred for 2h at that temperature and then, the solution was diluted with $CH_2Cl_2$ (15 ml) and washed with 0.1 N HCl (15 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure to afford **42** (318 mg, 100%) as a white solid that was used in subsequent reactions with no further purification.

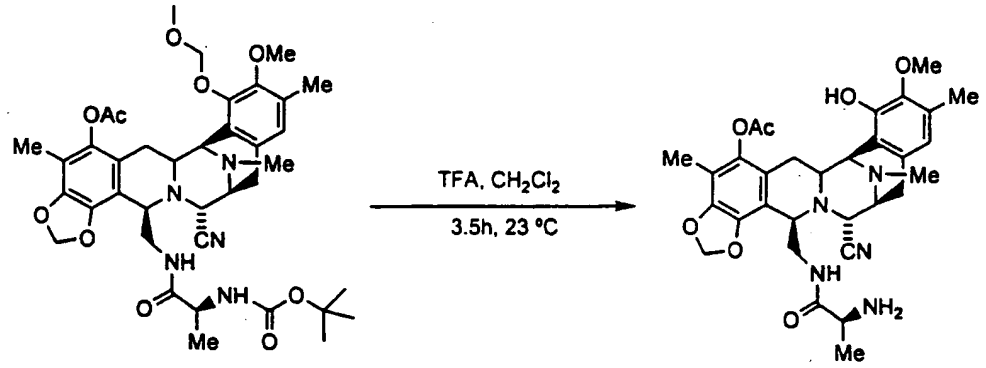
Rf: 0.5 (ethyl acetate:methanol 5:1).

$^1$H NMR (300 MHz, $CDCl_3$). δ 6.66 (s, 1H), 5.93 (d, *J*= 1.2 Hz, 1H), 5.83 (d, *J*= 1.2 Hz, 1H), 5.42 (t, *J*= 6.6 Hz, 1H), 5.07 (d, *J*= 5.7 Hz, 1H), 4.98 (d, *J*= 5.7 Hz, 1H), 4.16 (d, *J*= 1.8 Hz, 1H), 4.11 (d, *J*= 2.7 Hz, 1H), 3.98 (bs, 1H), 3.73-3.61 (m, 2H), 3.64 (s, 3H), 3.52-3.48 (m, 1H), 3.50 (s, 3H), 3.33 (d, *J*= 9.6 Hz, 1H), 3.17-3.14 (m, 1H), 2.97-2.87 (m, 1H), 2.75-2.70 (d, *J*= 16.8 Hz, 1H), 2.26 (s, 6H), 2.16 (s, 3H), 1.96 (s, 3H), 1.70 (dd, $J_1$= 11.7 Hz, $J_2$= 15.6 Hz, 1H), 1.33 (s, 9H), 0.59 (d, *J*= 6.0 Hz, 3H).

$^{13}$C NMR (75 MHz, $CDCl_3$) ): δ 172.0, 168.3, 162.3, 148.2, 144.4, 140.4, 140.2, 130.9, 130.5, 125.3, 123.4, 120.8, 117.6, 112.7, 111.7, 101.4, 99.1, 79.2, 59.5, 58.8, 57.5, 57.4, 56.4, 55.5, 55.0, 41.3, 39.0, 28.2, 26.4, 24.6, 19.9, 18.4, 15.4, 9.1.

ESI-MS m/z: Calcd. for $C_{38}H_{49}N_5O_{10}$: 735.82. Found $(M+H)^+$: 736.3.

Example 26




42 43

To a solution of **42** (318 mg, 0.432 ml) in $CH_2Cl_2$ (2.16 ml), trifluoroacetic acid (1.33 ml, 17.30 ml) was added and the reaction mixture was stirred for 3.5h at 23 °C. The reaction was quenched at 0 °C with saturated aqueous sodium bicarbonate (60 ml) and extracted with $CH_2Cl_2$ (2 x 70 ml). The combined organic layers were dried (sodium sulphate) and concentrated *in vacuo*. The residue was purified by flash column chromatography ($SiO_2$, ethyl acetate:methanol 20:1) to afford **43** (154 mg, 60%) as a white solid.

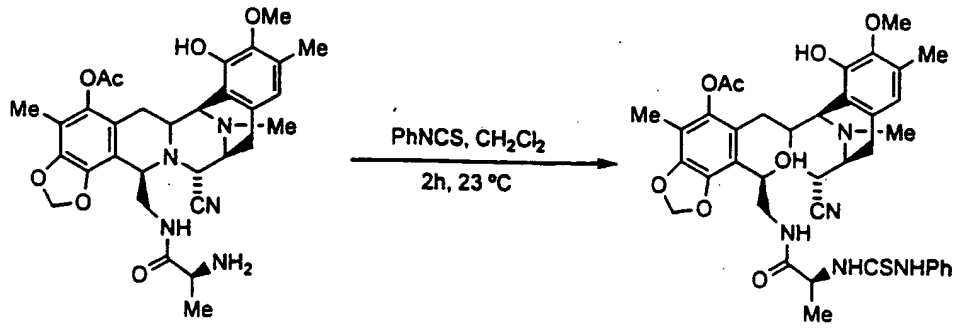
Rf: 0.22 (ethyl acetate:methanol 5:1).

$^1$H NMR (300 MHz, $CDCl_3$). δ 6.47 (s, 1H), 6.22 (bs, 1H), 5.95 (d, *J*= 1.2 Hz, 1H), 5.88 (d, *J*= 1.2 Hz, 1H), 4.08-4.06 (m, 2H), 4.01 (bs, 1H), 3.69 (s, 3H), 3.49 (d, *J*= 3.6 Hz, 1H), 3.33 (d, *J*= 8.1 Hz, 1H), 3.26-3.22 (m, 1H), 2.95 (dd, $J_1$= 8.1 Hz, $J_2$= 18 Hz, 1H), 2.80-2.76 (m, 2H), 2.58 (d, *J*=18Hz, 1H), 2.29 (s, 3H), 2.27 (s, 3H), 2.21 (s, 3H), 1.96 (s, 3H), 1.77 (dd, $J_1$= 12.3 Hz, $J_2$= 15.6 Hz, 1H), 0.90 (d, *J*=6.9 Hz, 3H).

$^{13}$C NMR (75 MHz, $CDCl_3$) ): δ 174.8, 169.0, 146.8, 144.4, 142.8, 140.5, 140.2, 131.1, 128.8, 120.8, 120.5, 117.1, 112.9, 111.6, 101.5, 60.3, 59.0, 56.5, 56.3, 55.6, 55.1, 50.2, 41.6, 39.5, 26.8, 26.3, 24.9, 20.2, 15.4, 9.2.

ESI-MS m/z: Calcd. for $C_{31}H_{37}N_5O_7$: 591.65. Found $(M+H)^+$: 592.3.

Example 27




43 44

To a solution of **43** (154 mg, 0.26 ml) in $CH_2Cl_2$ (1.3 ml), phenyl isothiocyanate (186 ml, 1.56 ml) was added and the mixture was stirred at 23° C for 2h. The reaction was concentrated *in vacuo* and the residue was purified by flash column chromatography ($SiO_2$, gradient Hexane to hexane:ethyl acetate 1:1) to afford **44** (120 mg, 63 %) as a white solid.

Rf: 0.41 (ethyl acetate:methanol 5:1).

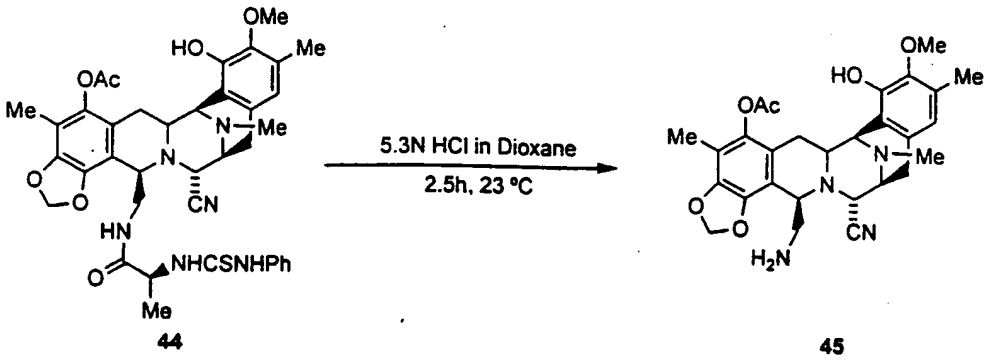
$^1$H NMR (300 MHz, $CDCl_3$). δ 8.17 (s, 1H), 7.49-7.44 (m, 3H), 7.31-7.24 (m, 3H), 7.05 (d, *J*= 6.9 Hz, 1H), 5.98 (d, *J*= 1.2 Hz, 1H), 5.87 (d, *J*= 1.2 Hz, 1H), 5.52 (bs, 1H), 4.54 (t, *J*= 6.6 Hz, 1H), 4.15 (d, *J*= 2.1 Hz, 1H), 4.03 (d, *J*= 2.7 Hz, 2H), 3.80 (bs, 1H), 3.66 (s, 3H), 3.40 (bs, 1H), 3.32 (d, *J*= 7.8 Hz, 1H), 3.16 (d, *J*= 11.7 Hz, 1H), 2.82-2.61 (m, 3H), 2.29 (s, 3H), 2.20 (s, 3H), 2.01 (s, 3H), 1.99 (s, 3H), 1.80 (dd, $J_1$= 12.0 Hz, $J_2$= 15.9 Hz, 1H), 0.62 (d, *J*=

6.0 Hz, 3H).

$^{13}$C NMR (75 MHz, $CDCl_3$) δ 178.5, 171.9, 168.7, 146.7, 144.5, 142.6, 140.6, 140.3, 136.3, 131.0, 129.9, 128.9, 126.7, 124.4, 120.9, 120.6, 117.7, 116.6, 112.7, 111.9, 101.4, 60.4, 58.7, 57.5, 56.1, 55.7, 55.1, 53.3, 41.4, 38.8, 26.3, 24.4, 20.2, 18.1, 15.3, 9.2.

ESI-MS m/z: Calcd. for $C_{38}H_{42}N_6O_7S$: 726.3. Found $(M+H)^+$: 727.3.

Example 28




44 45

To a solution of **44** (120 mg, 0.165 ml) in dioxane (0.9 ml), 5.3N HCl/dioxane (1.8 ml) was added and the reaction was stirred at 23 °C for 2.5h. Then, $CH_2Cl_2$ (10 ml) and $H_2O$ (5 ml) were added to this reaction and the organic layer was decanted. The aqueous phase was basified with saturated aq sodium bicarbonate (20 ml) (pH = 8) at 0 °C and then, extracted with $CH_2Cl_2$ (2x15 ml). The combined organic extracts were dried (sodium sulphate), and concentrated *in vacuo* to afford **45** (75 mg, 87%) as a white solid that was used in subsequent reactions with no further purification.

Rf: 0.23 (ethyl acetate:methanol 5:1).

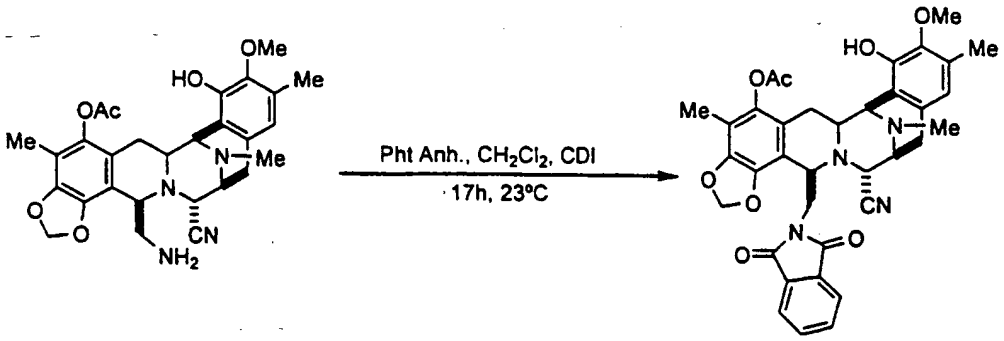
$^1$H NMR (300 MHz, $CDCl_3$): δ 6.43 (s, 1H), 5.94 (d, $J$= 1.2 Hz, 1H), 5.87 (d, $J$= 1.2Hz, 1H), 4.10 (d, $J$= 2.1 Hz, 1H), 3.98 (d, $J$= 2.4 Hz, 1H), 3.91 (bs, 1H), 3.69 (s, 3H), 3.34-3.25 (m, 2H), 3.05 (dd, $J_1$= 1.8 Hz, $J_2$= 8.1 Hz, 1H), 2.80-2.73 (m, 3H), 2.46 (d, $J$= 18 Hz, 1H), 2.30 (s, 3H), 2.28 (s,3H), 2.20 (s, 3H), 1.98 (s, 3H), 1.79 (dd, $J_1$= 12.6 Hz, $J_2$= 16.2 Hz, 1H);

$^{13}$C NMR (75 MHz, $CDCl_3$) ): δ 168.7, 146.7, 144.4, 142.9, 140.4, 130.4, 128.9, 121.1, 120.8, 117.8, 116.8, 113.6, 111.5, 101.4, 67.6, 60.5, 59.8, 58.4, 56.6, 55.8, 55.3, 43.6, 41.8, 31.3,

25.6, 20.2, 15.6, 9.2.

ESI-MS m/z: Calcd. for $C_{28}H_{32}N_4O_6$: 520.58. Found $(M+H)^+$: 521.3.

Example 29




45 Phtalascidin

To a solution of **45** (10 mg, 0.02 ml) in $CH_2Cl_2$ (0.4 ml) was added phthalic anhydride (2.84 mg, 0.02 ml) and the reaction mixture was stirred for 2 h at 23 °C. Then, carbonyldiimidazole (0.5 mg, 0.003 ml) was added and the mixture was stirred at 23 °C for 7h. Then, carbonyldiimidazole (2.61 mg, 0.016 ml) was added and the reaction was stirred at 23 °C for an additional 17h. The solution was diluted with $CH_2Cl_2$ (10 ml) and washed with 0.1 N HCl (5 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography (RP-18, $CH_3CN$:$H_2O$ 60:40) to afford phthalascidin (11.7 mg, 93%) as a white solid.

Rf: 0.37 ($CH_3CN$:$H_2O$ 7:3, RP-18).

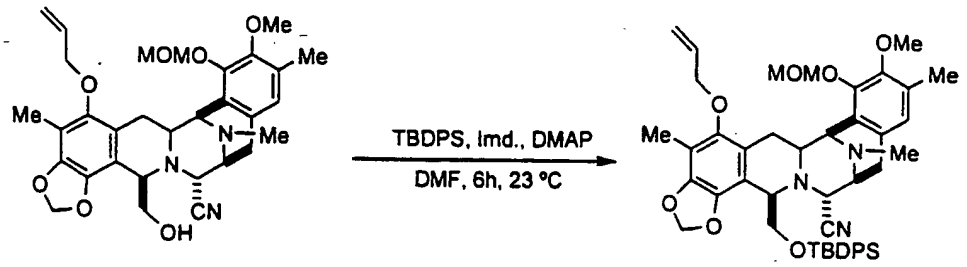
$^1$H NMR (300 MHz, $CDCl_3$): δ 7.72–7.68 (m, 2 h), 7.67-7.63 (m, 2 h), 6.38 (s, 1H), 5.69 (d, $J$= 1.2 Hz, 1H), 5.64 (d, $J$= 1.2 Hz, 1H), 5.30 (bs, 1H), 4.25-4.21 (m, 2 h), 4.02 (d, $J$= 2.1 Hz, 1H), 3.64-3.62 (m, 5H), 3.33 (d, $J$= 8.4 Hz, 1H), 3.21-3.16 (m, 1H), 3.02 (dd, $J_1$= 8.1 Hz, $J_2$= 18 Hz, 1H), 2.76 (dd, $J_1$= 1.8 Hz, $J_2$= 15.6 Hz, 1H), 2.63 (d, $J$= 17.7 Hz, 1H), 2.29 (s, 3H), 2.28 (s,3H), 2.21 (s, 3H), 2.0 (s, 3H), 1.73 (dd, $J_1$= 12.0 Hz, $J_2$= 15.3 Hz, 1H) );

$^{13}$C NMR (75 MHz, $CDCl_3$) ): δ 168.5, 167.6, 146.2, 144.2, 142.5, 141.0, 140.5, 133.4, 131.8, 130.7, 128.2, 120.9, 120.8, 117.9, 116.4, 113.6, 101.1, 60.4, 60.0, 57.0, 56.3, 55.6,

55.4, 41.6, 41.5, 26.5, 25.2, 20.2, 15.7, 9.4.

ESI-MS m/z: Calcd. for $C_{36}H_{34}N_4O_8$: 650. Found $(M+H)^+$: 651.2.

Example 30




25 26

To a solution of **25** (18 mg, 0.032 ml) in DMF (0.05 ml), cat. DMAP (0.5 mg, 0.004 ml), imidazole (5 mg, 0.08 ml) and *tert*-Butyldiphenylsilyl chloride (12.5 ml, 0.048 ml) were added at 0 °C and the reaction mixture was stirred for 6h at 23 °C. Water (10 ml) was added at 0 °C and the aqueous phase was extracted with hexane:ethyl acetate 1:10 (2 x 10 ml). The organic layer was dried (sodium sulphate), filtered, and the solvent was removed under reduced pressure. The crude was purified by flash column chromatography ($SiO_2$, hexane:ethyl acetate 3:1) to afford **26** (27 mg, 88 %) as a white solid.

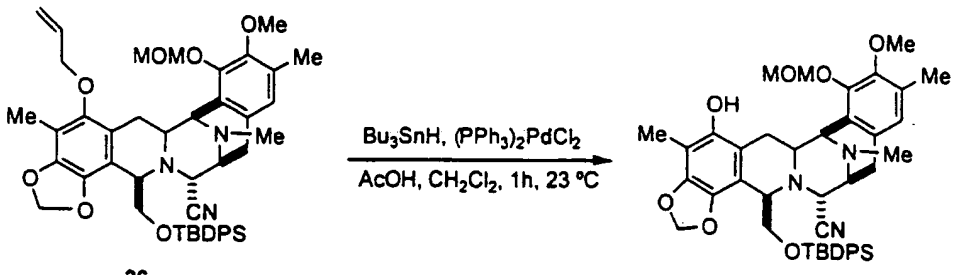
Rf: 0.29 (hexane:ethyl acetate 3:1).

$^1$H NMR (300 MHz, $CDCl_3$) δ 7.61-7.58 (m, 2 h), 7.42-7.28 (m, 8H), 6.71 (s, 1H), 6.19-6.02 (m, 1H), 5.78 (d, $J$= 1.2 Hz, 1H), 5.64 (d, $J$= 1.2 Hz, 1H), 5.40 (dd, $J_1$= 1.2 Hz, $J_2$= 17.1 Hz, 1H), 5.27 (dd, $J_1$= 1.2 Hz, $J_2$= 10.2 Hz, 1H), 5.13 (s, 2 h), 4.45 (d, $J$= 2.4 Hz, 1H), 4.24 (d, $J$= 2.1 Hz, 1H), 4.17-4.06 (m, 3H), 3.75 (s, 3H), 3.64 (dd, $J_1$= 2.4 Hz, $J_2$= 9.9 Hz, 1H), 3.59 (s, 3H), 3.42-3.21 (m, 4H), 3.10 (dd, $J_1$= 8.1 Hz, $J_2$= 17.7 Hz, 1H), 2.70 (d, $J$= 17.7 Hz, 1H), 2.33 (s, 3H), 2,26 (s, 3H), 2.11 (s, 3H), 2.08-1.89 (m, 1H), 0.87 (s, 9H);

$^{13}$C NMR (75 MHz, $CDCl_3$): δ 148.5, 148.3, 148.1, 144.0, 139.0, 135.6, 135.4, 133.8, 133.1, 132.6, 130.5, 130.3, 129.6, 129.4, 127.5, 127.4, 125.1, 124.3, 121.6, 118.5, 117.5, 112.9, 111.7, 100.8, 99.2, 74.0, 67.7, 61.5, 59.6, 59.0, 57.7, 57.1, 55.4, 41.6, 29.6, 26.6, 25.5, 18.8, 15.8, 9.2.

ESI-MS m/z: Calcd. for $C_{47}H_{55}N_3O_7Si$: 801.3. Found $(M+H)^+$: 802.3.

Example 31




26 ET-11

To a solution of **26** (7 mg, 0.0087 ml) in $CH_2Cl_2$ (0.15 ml), acetic acid (2.5 ml, 0.044 ml), $(PPh_3)_2PdCl_2$ (0.5 mg, $6.96 \times 10^{-4}$ ml) and $Bu_3SnH$ (3.5 ml, 0.013 ml) were added at 23 °C. The reaction mixture was stirred at that temperature for 1h. The solution was diluted with a mixture of hexane:ethyl acetate 5:1 (0.5 ml) and poured into a pad of flash column ($SiO_2$, gradient 5:1 to 1:1 hexane:ethyl acetate) affording **ET-11** (5 mg, 75 %) as a white solid.

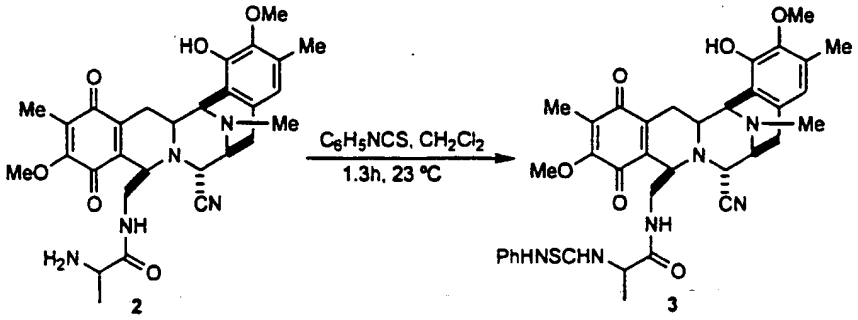
Rf: 0.36 (hexane:ethyl acetate 1:5, silica).

$^1$H NMR (300 MHz, $CDCl_3$): δ 7.56 (m, 2 h), 7.41-7.25 (m, 8H), 6.67 (s, 1H), 5.72 (d, $J$= 1.0 Hz, 1H), 5.58 (d, $J$= 1.0 Hz, 1H), 5.51 (s, 1H), 5.38 (d, $J$= 5.75 Hz, 1H), 5.16 (d, $J$= 5.7 Hz, 1H), 4.57 (d, $J$= 2.9 Hz, 1H), 4.21 (m, 1H), 4.09 (m, 1H), 3.72 (s, 3H), 3.71 (s, 3H), 3.68 (dd, $J_1$= 2.1 Hz, $J_2$= 10.4 Hz, 1H), 3.38-3.26 (m, 3H), 3.11 (dd, $J_1$= 2.5 Hz, $J_2$= 15.7 Hz, 1H), 3.01 (dd, $J_1$= 8.9 Hz, $J_2$= 17.9 Hz, 1H), 2.70 (d, $J$= 17.9 Hz, 1H), 2.31 (s, 3H), 2.25 (s, 3H), 2.06 (s, 3H), 1.89 (dd, $J_1$= 12.1 Hz, $J_2$= 15.7 Hz, 1H), 0.9 (s, 9H). );

$^{13}$C NMR (75 MHz, $CDCl_3$): δ 149.0, 147.4, 145.3, 144.3, 136.3, 135.7, 135.4, 133.2, 130.9, 130.5, 129.6, 129.5, 127.5, 125.0, 118.6, 112.5, 112.1, 105.7, 100.5, 99.8, 68.5, 61.5, 59.7, 58.8, 57.7, 56.9, 56.5, 55.4, 41.7, 26.6, 26.2, 25.5, 18.9, 15.8, 14.2, 8.7.

ESI-MS m/z: Calcd. for $C_{44}H_{51}N_3O_7Si$: 761. Found $(M+H)^+$: 762.

Example 32




A solution of **2** (3.0 g, 5.46 ml) and phenyl isothiocyanate (3.92mL, 32.76 ml) in $CH_2Cl_2$ (27 ml) was stirred at 23° C for 1.5h. The reaction mixture was partitioned between $CH_2Cl_2$ (10 ml) and $H_2O$ (5 ml). The organic layer was dried over sodium sulphate, filtered and concentrated. The residue was purified by flash column chromatography ($SiO_2$, gradient Hex to 2:3 hexane:ethyl acetate) to give **3** (3.29 g, 88%) as a yellow solid.

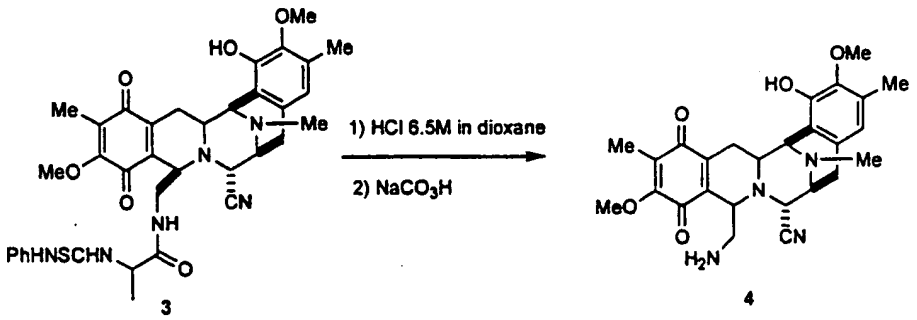
Rf: 0.27 (ACN:$H_2O$ 3:2, RP-C18);

$^1$H NMR (300 MHz, $CDCl_3$): δ 7.77 (bs, 1H), 7.42-7.11 (m, 5H), 6.65 (d, 1H), 6.29 (s, 1H), 5.6-5.5 (m, 1H), 4.19-4.14 (m, 2 h), 4.08 (d, 1H), 3.92 (s, 3H), 3.87-3.65 (m, 6H), 3.77 (s, 3H), 3.37-2.98 (m, 8H), 2.50 (d, 1H), 2.31 (s, 3H), 2.20 (s, 3H), 1.96 (d, 1H), 1.87 (s, 3H), 1.81-1.75 (m, 1H), 0.96 (d, 3H);

$^{13}$C NMR (75 MHz, $CDCl_3$):δ 185.7, 180.9, 178.9, 172.0, 155.7, 147.1, 143.2, 142.4, 136.0, 135.1, 130.5, 129.9, 129.3, 128.5, 126.9, 124.4, 120.2, 117.4, 116.3, 77.1, 60.9, 58.6, 56.2, 55.8, 55.0, 54.6, 53.5, 41.7, 40.3, 25.1, 24.5, 18.4, 15.8, 8.7

ESI-MS m/z: Calcd. for $C_{36}H_{40}N_6O_6S$: 684.8. Found $(M+H)^+$: 685.2.

Example 33



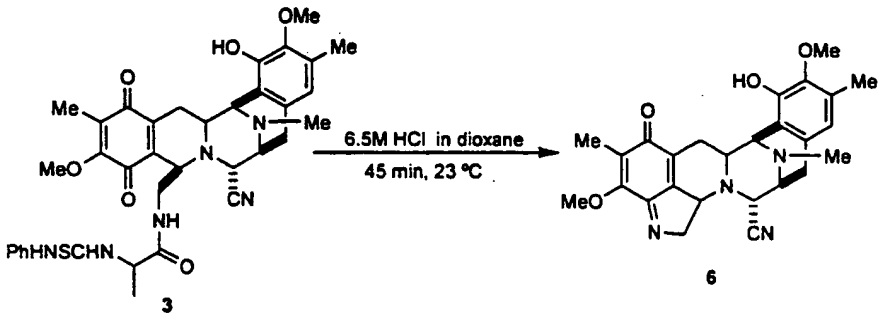
A solution of **3** (0.143 g, 0.208 ml) in 6.5 M HCl/dioxane (150 ml) was stirred at 23 °C for 6h. Then, toluene (3 ml) was added to this reaction and the organic layer was decanted. The residue was partitioned between saturated aqueous sodium bicarbonate (3 ml) and $CHCl_3$ (3x3 ml) The organic layers were dried and concentrated to afford title compound as a mixture of **4** and **6** (**4:6** 90:10) which slowly cyclizes to **6** on standing.

Rf: 0.4 (ethyl acetate:methanol5:1, silica);

$^1$H NMR (300 MHz, $CDCl_3$): δ 6.45 (s, 1H), 4.16 (m, 1H), 4.02 (d, 1H), 3.96 (s, 3H), 3.79 (m, 2 h), 3.75 (s, 3H), 3.35 (m, 1H), 3.20-3.00 (m, 3H), 2.87 (d, 1H), 2.75 (d, 1H), 2.43 (d, 1H), 2.34 (s, 3H), 2.30 (s, 3H), 1.93 (s, 3H), 1.72-1.5 (m, 3H);

ESI-MS m/z: Calcd. for $C_{26}H_{30}N_4O_5$: 478.5. Found $(M+H)^+$: 479.2

Example 34




A solution of **3** (0.143 g, 0.208 ml) in 6.5M HCl/dioxane (150 ml) was stirred at 23 °C for 1h. Evaporation of the solvent gave a residue which was purified by flash column chromatography (ethyl acetate/methanol/triethylamine 100:25:0.1) to give **6** (80 mg, 83%) as a yellow solid.

Rf: 0.26 (ACN:$H_2O$ 3:2, RP-C18);

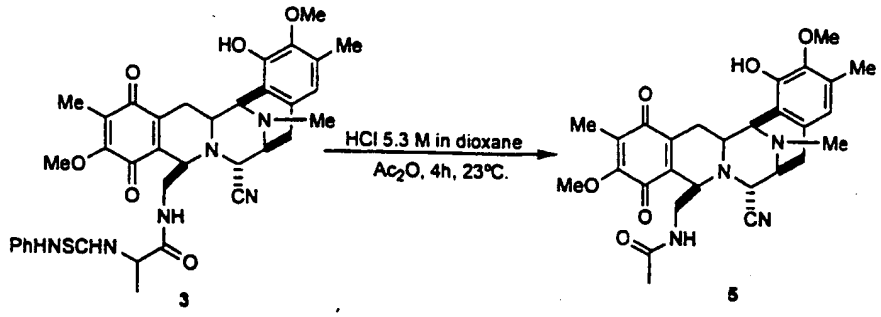
$^1$H NMR (500 MHz, $CDCl_3$): δ 6.46 (s, 1H), 5.9 (bs, 1H) 4.67 (dd, *J*=18.3 Hz, *J*= 7.8 Hz, 1H), 4.24 (d, 1H), 4.16 (s, 3H), 3.93 (d, *J*=2.7 Hz, 1H), 3.8 (m, 2 h), 3.77 (s, 3H), 3.45 (m, 2 h), 3.08 (dd, *J*=17.9 Hz, *J*=3.6 Hz, 1H), 2.78 (m, 1H), 2.55 (d, 1H), 2.3 (m, 1H), 2.3 (s, 3H), 2.

28 (s, 3H), 1.90 (s, 3H);

$^{13}C$ NMR (75 MHz,$CDCl_3$):δ 186.2, 162.1, 154.9, 146.9, 145.3, 143.0, 130.1, 129.4, 128.1, 125.0, 121.4, 116.4, 116.2, 66.6, 60.7, 60.7, 60.1, 59.6, 58.8, 55.6, 54.9, 41.9, 25.3, 24.7, 15.7, 8.9.

ESI-MS m/z: Calcd. for $C_{26}H_{28}N_4O_4$: 460.5. Found $(M+H)^+$: 461.1

Example 35




To a solution of **3** (2.38 g, 3.47 ml) in dioxane (5 ml) 5.3M HCl in dioxane (34 ml) was added and the reaction was stirred at 23 °C for 45 minutes. Then $Ac_2O$ (51 ml, 539.5 ml) was added and the mixture was stirred for 4h. The reaction was cooled at 0 °C and partitioned between aqueous saturated $Na_2CO_3$ (300 ml) and ethyl acetate (300 ml) at this temperature. The organic phase was dried over sodium sulphate, filtered and concentrated. The residue was purified by flash column chromatography ($SiO_2$, gradient $CH_2Cl_2$ to $CH_2Cl_2$:ethyl acetate 1:2) to give **5** (1.75 g, 97%) as a yellow solid.

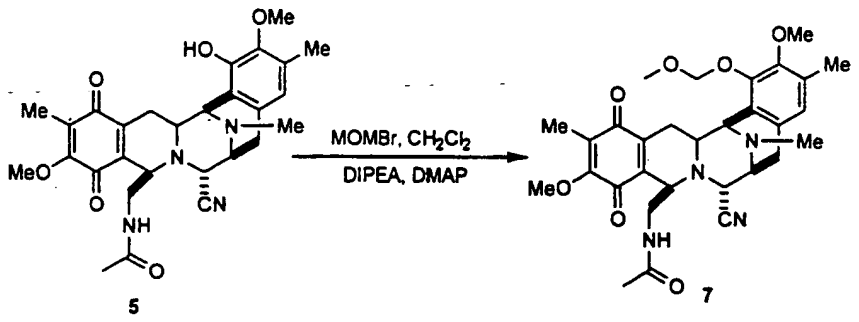
Rf: 0.53 (ACN:$H_2O$ 3:2, RP-C18);

$^1H$ NMR (300 MHz, $CDCl_3$): δ 6.51 (s, 1H), 5.98 (bs, 1H), 4.84 (dd, 1H), 4.17 (d, 1H), 4.00 (d, 1H), 3.99 (s, 3H), 3.85 (bs, 1H), 3.81 (m, 1H), 3.74 (s, 3H), 3.70 (d, 1H), 3.23 (m, 1H), 3.11 (dd, 1H), 3.09 (m, 1H), 2.93 (m, 2 h), 2.44 (d, 1H), 3.67 (s, 3H), 2.25 (s, 3H), 1.70 (s, 3H), 1.60-1.50 (m, 2 h), 1.29 (s, 3H);

$^{13}C$ NMR (75 MHz, $CDCl_3$): δ 185.9, 180.8, 169.9, 160.2, 156.2, 147.0, 143.1, 140.4, 136.1, 130.6, 129.6, 127.9, 120.4, 117.2, 61.0, 60.7, 58.6, 56.1, 55.7, 55.1, 54.3, 41.8, 41.1, 25.7, 23.9, 22.2, 15.7, 8.7.

ESI-MS m/z: Calcd. for $C_{28}H_{32}N_4O_6$: 520.6. Found $(M+H)^+$: 521.1

Example 36




5 7

To a solution of **5** (1.75 g, 3.36 ml) in $CH_2Cl_2$ (17 ml) diisopropylethylamine (11.71 ml, 67.23 ml), DMAP (20 mg, 0.17 ml) and bromomethyl methyl ether (4.11 ml, 50.42 ml) were added at 0 °C. After 6 h at 23 °C the reaction was partitioned between $CH_2Cl_2$ (50 ml) and aqueous saturated sodium bicarbonate (25 ml). The organic layer was dried over sodium sulphate and the solvent was eliminated under reduced pressure. The crude was purified by flash column chromatography (RP-18, $CH_3CN/H_2O$ 1/1) to give **7** (1.32 g, 70%) as a yellow solid.

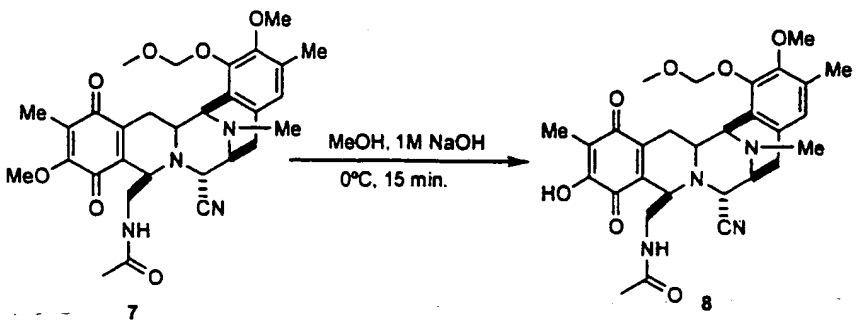
Rf: 0.34 (ACN:$H_2O$ 2:3, RP-C18);

$^1H$ NMR (300 MHz, $CDCl_3$): δ 6.74 (s, 1H), 5.14 (s, 2 h), 4.82 (m, 1H), 4.22 (d, 1H), 4.00 (s, 3H), 4.0 (m, 1H), 3.83 (m, 2 h), 3.7 (s, 3H), 3.58 (s, 3H), 3.4 (m, 1H), 3.2-2.95 (m, 6H), 2.43 (d, 1H), 2.37 (s, 3H), 2.22 (s, 3H), 1.89 (s, 3H), 1.5-1.4 (m, 2 h), 1.31 (s, 3H);

$^{13}C$ NMR (75 MHz, $CDCl_3$): δ 185.9, 180.7, 169.6, 156.2, 148.9, 148.5, 140.3, 136.2, 131.3, 130.1, 127.7, 124.6, 123.7, 117.3, 99.5, 99.2, 60.9, 59.7, 58.8, 57.7, 56.4, 55.7, 55.0, 54.2, 51.0, 41.6, 41.0, 40.5, 25.5, 23.9, 22.3, 19.3, 15.6, 14.6, 8.6.

ESI-MS m/z: Calcd. for $C_{30}H_{36}N_4O_7$: 564.6. Found $(M+H)^+$: 565.3

Example 37




7 8

To a solution of 7 (0.37 g, 0.65 ml) in methanol (74 ml) at 0 °C was added 1M sodium hydroxide (130 ml). The reaction was stirred for 15 minutes and then, quenched at 0 °C with 6M HCl to pH = 5. The mixture was extracted with ethyl acetate (3 x 50 ml) and the combined organic layers were dried over sodium sulphate and concentrated *in vacuo*. The residue was purified by flash column chromatography (RP-C18 $CH_3CN$:$H_2O$ 1/:1) to afford **8** (232 mg, 65%) as a yellow oil.

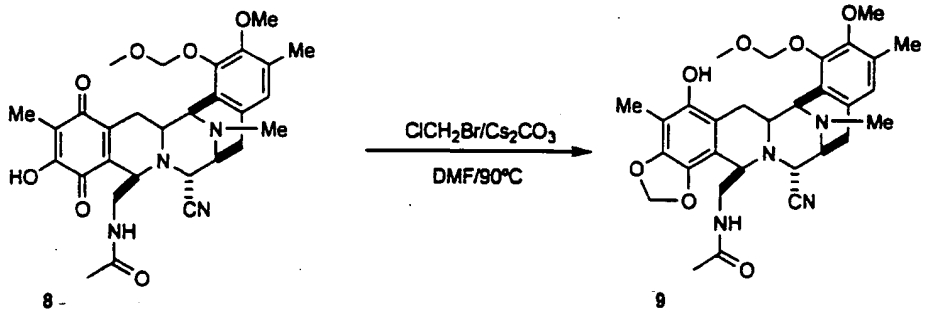
Rf: 0.5 (ACN:$H_2O$ 3:2, RP-C18);

$^1$H NMR (300 MHz, $CDCl_3$): δ 6.75 (s, 1H), 5.15 (s, 2 h), 4.86 (m, 1H), 4.26 (d, 1H), ), 4.01 (d, 1H), 3.88-3.81 (m, 2 h), 3.70 (s, 3H), 3.58 (s, 3H), 3.39 (m, 1H), 3.27-3.21 (m, 1H), 3.18-3.08 (m, 2 h), 3.03-2.97 (m, 1H) 2.47 (d, 1H), 2.37 (s, 3H), 2. 22 (s, 3H), 1.90 (s, 3H), 1.57-1.46 (m, 2 h), 1.33 (s, 3H);

$^{13}$C NMR (75 MHz, $CDCl_3$): δ 185.3, 180.6, 175.9, 170.1, 151.5, 148.9, 148.6, 143.3, 133.7, 131.5, 129.9, 124.7, 123.5, 117.1, 117.0, 99.2, 59.8, 58.7, 57.8, 56.3, 55.3, 54.9, 54.3, 41.5, 40.7, 29.6, 25.5, 24.4, 22.2, 20.7, 15.7, 8.0.

ESI-MS m/z: Calcd. for $C_{29}H_{34}N_4O_7$: 550.6. Found $(M+H)^+$: 551.2

Example 38




8 9

To a degassed solution of compound **8** (240mg, 0.435 ml) in DMF (30 ml) 10 % Pd/C (48 mg) was added and the reaction was stirred under $H_2$ (atmospheric pressure.) for 1h. The reaction was filtered through a pad of celite under Argon to a Schlenk tube, as a colourless solution, containing anhydrous $Cs_2CO_3$ (240 mg, 0.739 ml). Then, bromochloromethane (0.566 ml, 8.71 ml) was added. The tube was sealed and stirred at 90 °C for 3h. The reaction was cooled and filtrated through celite and washed with $CH_2Cl_2$. The organic layer was concentrated and dried (sodium sulphate) to afford **9** as a brown oil that was used in the next step with no further purification.

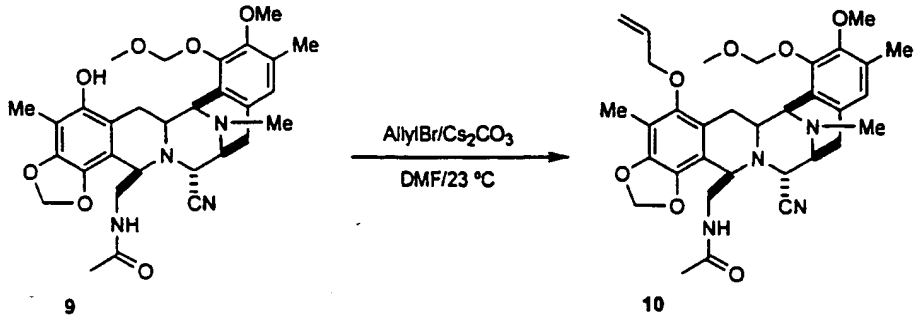
Rf: 0.36 ($SiO_2$, hexane:ethyl acetate 1:5)

$^1$H NMR (300 MHz, $CDCl_3$): δ 6.71 (s, 3H), 5.89 (d, 1H), 5.81 (d, 1H), 5.63 (bs, 1H), 5.33 (d, 1H), 5.17 (d, 1H), 4.97 (m, 1H), 4.20 (d, 1H), 4.09 (m, 1H), 3.99 (m, 1H), 3.68 (m, 1H), 3.65 (s, 6H), 3.59-3.47 (m, 4H), 3.37-3.27 (m, 2 h), 3.14- 2.97 (m, 2 h), 2.62 (d, 1H), 2.32 (s, 3H), 2.20 (s, 3H), 2.08 (s, 3H), 1.72 (m, 1H), 1.36 (s, 3H);

$^{13}$C NMR (75 MHz, $CDCl_3$): δ 169.8, 149.1, 147.4, 145.5, 136.2, 130.9, 130.8, 125.0, 122.9, 117.7, 112.6, 111.8, 106.4, 100.8, 99.8, 59.8, 58.9, 57.7, 56.6, 56.4, 55.5, 55.2, 41.6, 40.1, 29.6, 25.9, 25.0, 22.6, 15.6, 8.8.

ESI-MS m/z: Calcd. for $C_{30}H_{36}SiN_4O_7$: 564.6. Found $(M+H)^+$: 565.3.

Example 39




9 10

To a flask containing **9** (245 mg, 0.435 ml) in DMF, (4 ml), cesium carbonate (425 mg, 1.30 ml) and allyl bromide (376 ml, 4.35 ml) were added at 0 °C and the mixture was stirred at 23 °C for 1h. The reaction was filtered though a pad of celite and partitioned between $CH_2Cl_2$ (25 ml) and $H_2O$ (10 ml). The organic phase was dried (sodium sulphate) and concentrated at reduced pressure to afford a residue that was purified by flash column chromatography ($SiO_2$, $CHCl_3$:ethyl acetate 1:2) to give **10** as a yellow oil. (113 mg, 43 %).

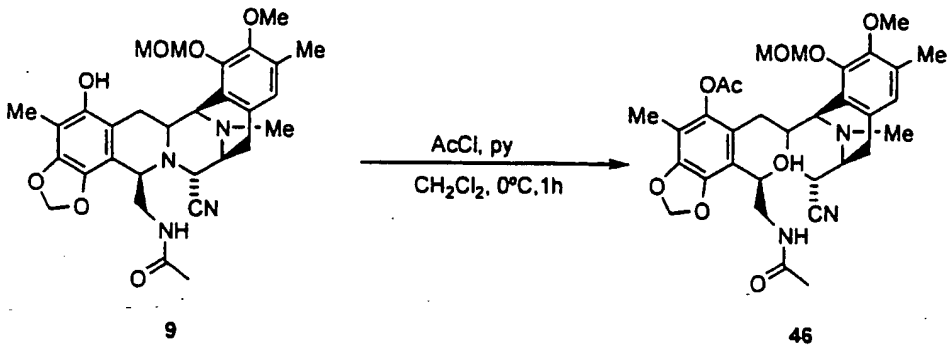
Rf: 0.36 (hexane:ethyl acetate 1:5)

$^1$H NMR (300 MHz, $CDCl_3$): δ 6.74 (s, 1H), 6.3-6.0 (m, 1H), 5.94 (d, 1H), 5.87 (d, 1H), 5.43-5.36 (m, 2 h), 5.22 (s, 2 h), 5.00 (m, 1H), 4.22 (m, 1H), 4.17-4.01 (m, 1H), 3.98 (m, 2 h), 3.71-3.67 (m, 1H), 3.69 (s, 3H), 3.62-3.51 (m, 3H), 3.58 (s, 3H), 3.39-3.37 (m, 1H), 3.31-3.26 (m, 3H), 3.09 (dd, 1H), 2.56 (d, 1H), 2.36 (s, 3H), 2.21 (s, 3H), 2.11 (s, 3H), 2.24-2.10 (m, 1H), 1.82-1.73 (m, 1H), 1.24 (bs, 3H)

$^{13}$C NMR (75 MHz, $CDCl_3$): δ 169.4, 148.8, 148.3, 139.1, 133.7, 130.9, 130.3, 125.2, 120.2, 117.7, 113.1, 112.6, 101.3, 99.3, 74.1, 59.7, 59.3, 57.8, 57.0, 56.1, 56.1, 55.2, 41.6, 41.0, 40.9, 29.7, 26.3, 22.5, 15.6, 9.3

ESI-MS m/z: Calcd. for $C_{33}H_{40}N_4O_7$: 604.7. Found $(M+H)^+$: 605.3.

Example 40




9 46

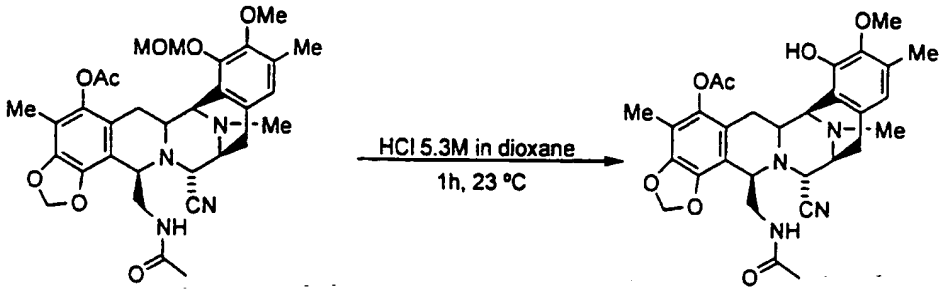
To a solution of **9** (22 mg, 0.039 ml) in $CH_2Cl_2$ (0.2 ml), acetyl chloride (2.79 ml, 0.039 ml) and pyridine (3.2 ml, 0.039 ml) were added at 0 °C. The reaction mixture was stirred for 1h and then, the solution was diluted with $CH_2Cl_2$ (10 ml) and washed with 0.1 N HCl (5 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure to afford **46** (22 mg, 93%) as a white solid.

Rf: 0.4 (hexane:ethyl acetate 1:5).

$^1$H NMR (300 MHz, $CDCl_3$). δ 6.74 (s, 1H), 5.97 (d, *J*= 0.9 Hz, 1H), 5.91 (d, *J*= 0.9 Hz, 1H), 5.12 (d, *J*= 5.7 Hz, 2 h), 5.04 (d, *J*= 5.7 Hz, 1H) 4.90 (t, *J*= 6 Hz, 1H), 4.17 (d, *J*= 2.7 Hz, 1H), 4.05 (d, *J*= 2.7 Hz, 1H), 4.01 (bs, 1H), 3.71 (s, 3H), 3.57 (s, 3H), 3.50-3.44 (m, 2 h), 3.38-3.36 (m, 1H), 3.30-3.26 (m, 1H), 3.00 (dd, $J_1$= 7.8 Hz, $J_2$= 18.0 Hz, 1H), 2.79 (d, *J*= 12.9 Hz, 1H), 2.60 (d, *J*=18.0 Hz, 1H), 2.35 (s, 3H), 2.32 (s, 3H), 2.21 (s, 3H), 2.00 (s, 3H), 1.68 (dd, $J_1$=11.7 Hz, $J_2$= 15.6 Hz, 1H).

ESI-MS m/z: Calcd. for $C_{32\,h38}N_4O_8$: 606.67. Found $(M+H)^+$: 607.3.

Example 41




46 47

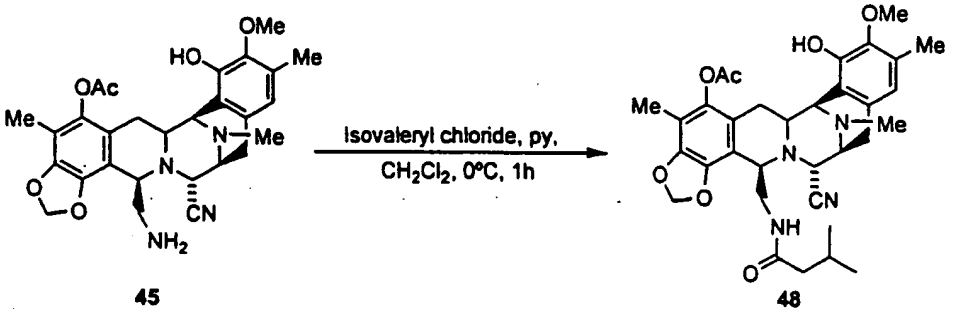
To a solution of **46** (8 mg, 0.013 ml) in dioxane (0.1 ml), 5.3N HCl/dioxane (0.5 ml) was added and the reaction was stirred at 23 °C for 1h. Then, the solution was diluted with $CH_2Cl_2$ (5 ml) and washed with 0.1 N HCl (3 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure to afford **47** (5 mg, 70%) as a white solid.

Rf: 0.4 (hexane:ethyl acetate 1:5).

$^1$H NMR (300 MHz, $CDCl_3$). δ 6.51 (s, 1H), 5.97 (d, *J*= 1.2 Hz, 1H), 5.91 (d, *J*= 1.2 Hz, 1H), 4.97 (bs, 1H), 4.11 (bs, 1H), 4.04-4.02 (m, 2 h), 3.75 (s, 3H), ), 3.65 (d, *J*= 2.1 Hz, 2 h), 3.56-3.30 (m, 2 h), 3.04 (dd, $J_1$= 7.5 Hz, $J_2$= 18 Hz, 1H), 2.80 (d, *J*= 14.4 Hz, 1H), 2.59 (d, *J*= 18.3 Hz, 1H), 2.33 (s, 3H), 2.24 (s, 3H), 2.00 (s, 3H), 1.76 (dd, $J_1$= 12.0 Hz, $J_2$= 15.9 Hz, 1H), 1.33 (s, 3H), 1.25 (s, 3H).

ESI-MS m/z: Calcd. for $C_{30}H_{34}N_4O_7$: 562.61. Found $(M+H)^+$: 563.3.

Example 42




45 48

To a solution of **45** (10 mg, 0.0192 ml) in $CH_2Cl_2$ (0.3 ml), isovaleryl chloride (2.34 ml,

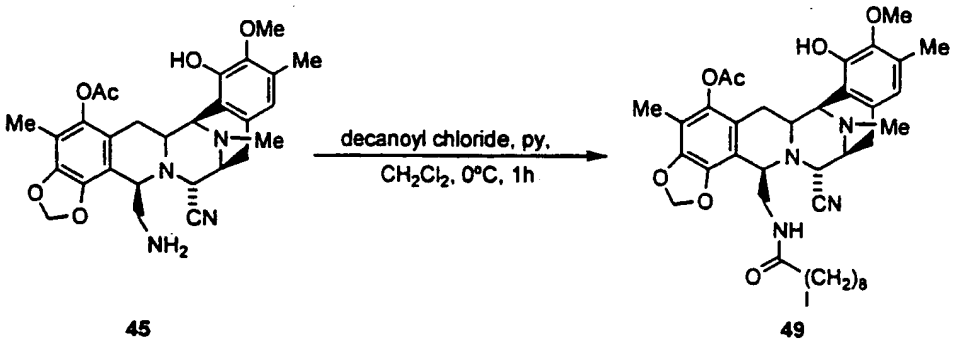
0.0192 ml) and pyridine (1.55 ml, 0.0192 ml) were added at 0 °C. The reaction mixture was stirred for 1h and then, the solution was diluted with $CH_2Cl_2$ (5 ml) and washed with 0.1 N HCl (3 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography ($SiO_2$, Hex: ethyl acetate 1:2) to afford **48** (11 mg, 95%) as a white solid.

Rf: 0.12 (Hex: ethyl acetate 1:2).

$^1$H NMR (300 MHz, $CDCl_3$): δ 6.50 (s, 1H), 5.98 (d, *J*= 1.5Hz, 1H), 5.91(d, *J*= 1.5 Hz, 1H), 5.75 (s, 1H), 5.02 (t, *J*= 5.4 Hz, 1H), 4.10 (d, *J*= 1.5 Hz, 1H), 4.06 (d, *J*= 2.7 Hz, 1H), 4.02 (d, *J*= 2.7 Hz, 1H), 3.77 (s, 3H), 3.76-3.71 (m, 1H), 3.86-3.28 (m, 3H), 3.04 (dd, $J_1$= 8.1 Hz, $J_2$= 18.3Hz, 1H), 2.78 (d, *J*=15.9 Hz, 1H), 2.55 (d, *J*=18 Hz, 1H), 2.32 (s, 6H), 2.26 (s, 3H), 1.98 (s, 3H), 1.84-1.68 (m, 2 h), 1.36 (d, *J*= 7.2 Hz, 2 h), 0.69 (d, *J*= 6.6 Hz, 3H), 0.62 (d, *J*=6.6 Hz, 3H).

ESI-MS m/z: Calcd. for $C_{33}H_{40}N_4O_7$: 604.69. Found $(M+H)^+$: 605.3.

Example 43




**45** **49**

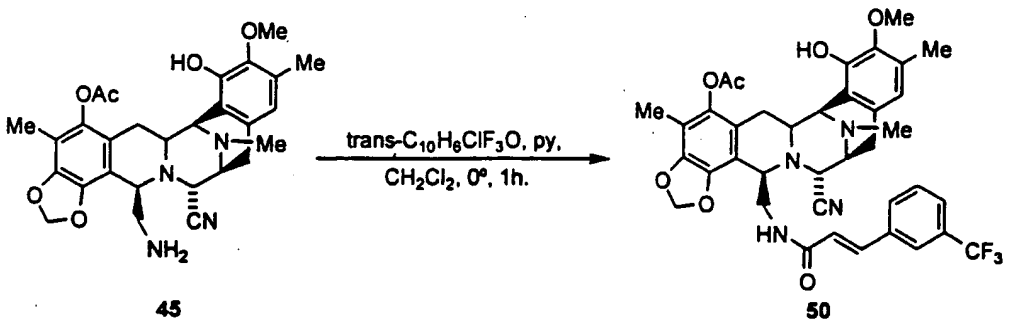
To a solution of **45** (10 mg, 0.0192 ml) in $CH_2Cl_2$ (0.3 ml), isovaleryl chloride (3.98 ml, 0.0192 ml) and pyridine (1.55 ml, 0.0192 ml) were added at 0 °C. The reaction mixture was stirred for 1h and then, the solution was diluted with $CH_2Cl_2$ (5 ml) and washed with 0.1 N HCl (3 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography ($SiO_2$, Hex: ethyl acetate 1:2) to afford **49** (12.4 mg, 96%) as a white solid.

Rf: 0.7 (ethyl acetate:methanol10:1).

$^1$H NMR (300 MHz, $CDCl_3$): δ 6.50 (s, 1H), 5.98 (d, *J*= 1.5Hz, 1H), 5.91 (d, *J*= 1.5 Hz, 1H), 5.73 (s, 1H), 5.08 (t, *J*= 5.4 Hz, 1H), 4.10 (d, *J*= 1.5 Hz, 1H), 4.05 (m., 1H), 4.01 (m, 1H), 3.76 (s, 3H), 3.65-3.61 (m, 1H), 3.40-3.27 (m, 3H), 3.03 (dd, $J_1$= 8.1 Hz, $J_2$= 18.6 Hz, 1H), 2.78 (d, *J*=13.2 Hz, 1H), 2.57 (d, *J*=18.3 Hz, 1H), 2.32 (s, 3H), 2.31 (s, 3H), 2.25 (s, 3H), 1.99 (s, 3H), 1.79 (dd, $J_1$= 12.0 Hz, $J_2$= 16.5 Hz, 1H), 1.73-1.42 (m, 4H), 1.33-1.18 (m, 10H), 1.03 (m, 2 h), 0.87 (t, *J*= 6.6 Hz, 3H).

ESI-MS m/z: Calcd. for $C_{38}H_{50}N_4O_7$: 674.83. Found $(M+H)^+$: 675.5.

Example 44




45 50

To a solution of **45** (14.5 mg, 0.0278 ml) in $CH_2Cl_2$ (0.3 ml), trans-3-trifluoromethyl cinnamoyl chloride (4.76 ml, 0.0278 ml) and pyridine (2.25 ml, 0.0278 ml) were added at 0 °C. The reaction mixture was stirred for 1h and then, the solution was diluted with $CH_2Cl_2$ (5 ml) and washed with 0.1 N HCl (3 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography ($SiO_2$, Hex: ethyl acetate 1:1) to afford **50** (18.7 mg, 94%) as a white solid.

Rf: 0.64 (ethyl acetate:methanol5:1).

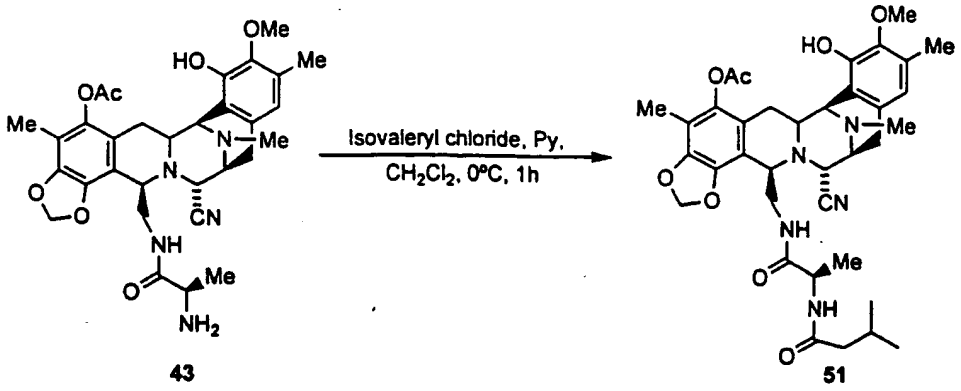
$^1$H NMR (300 MHz, $CH_3OD$). δ 7.74-7.55 (m, 4H), 7.23 (d, *J*= 16.0 Hz, 1H), 6.34 (s, 1H), 6.12 (d, *J*= 16.0 Hz, 1H), 6.07 (d, *J*= 0.9 Hz, 1H), 5.96 (d, *J*= 0.9 Hz, 1H), 4.39 (d, *J*= 2.4 Hz, 1H), 4.07-4.05 (m, 1 H), 3.81 (bs, 1H), 3.46-3.51 (m, 3H), 3.42 (s, 3H), 3.09 (br d, *J*= 12.0 Hz, 1H), 2.94-2.85 (m, 2 h), 2.74 (d, *J*=18.3 Hz, 1H), 2.38 (s, 3H), 2.23 (s, 3H), 2.02 (s, 3H),

1.80 (s, 3H), 1.84-1.75 (m, 1H).

$^{13}$C NMR (75 MHz, $CDCl_3$) ): δ 168.7, 165.3, 146.5, 144.7, 142.6, 140.6, 138.0, 135.9, 131.0, 130.9, 129.1, 128.6, 125.8, 125.7, 124.5, 124.4, 122.7, 121.2, 117.8, 116.5, 113.0, 112.0, 101.7, 60.4, 59.1, 56.5, 56.4, 55.6, 55.3, 41.8, 40.3, 26.6, 25.1, 20.3, 15.4, 9.3.

ESI-MS m/z: Calcd. for $C_{38}H_{37}F_3N_4O_7$: 718.72. Found $(M+H)^+$: 719.3.

Example 45




43 51

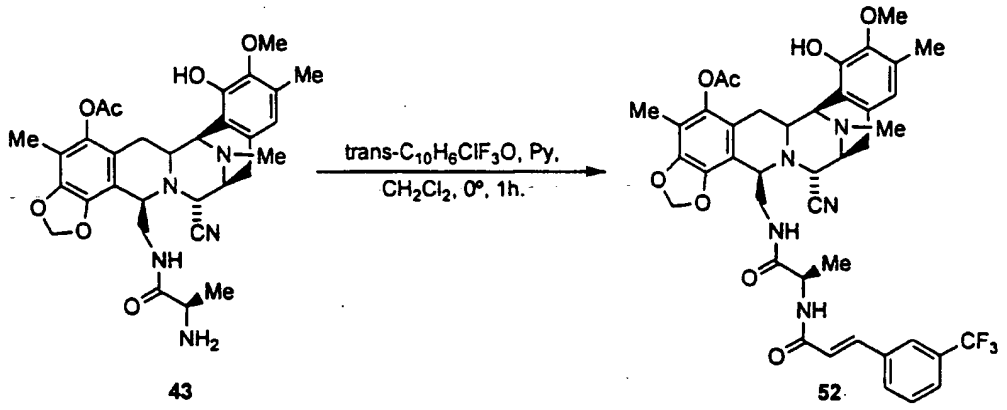
To a solution of **43** (33 mg, 0.0557 ml) in $CH_2Cl_2$ (0.4 ml), isovaleryl chloride (6.79 ml, 0.0557 ml) and pyridine (4.5 ml, 0.0557 ml) were added at 0 °C. The reaction mixture was stirred for 1h and then, the solution was diluted with $CH_2Cl_2$ (5 ml) and washed with 0.1 N HCl (3 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography ($SiO_2$, Hex: ethyl acetate 1:2) to afford **51** (34 mg, 91%) as a white solid.

Rf: 0.09 (Hex: ethyl acetate 1:2).

$^1$H NMR (300 MHz, $CDCl_3$): δ 6.46 (s,1H), 6.10 (bs, 1H), 5.99 (d, *J*= 0.9Hz, 1H), 5.90 (d, *J*= 0.9 Hz, 1H), 5.30 (t, *J*= 6.0 Hz, 1H), 4.10-4.05 (m, 3H),3.81 (bs, 1H), 3.74 (s, 3H), 3.54 (bs,1H), 3.38-3.36 (m, 1H), 3.29-3.21 (m, 1H), 3.00 (dd, $J_1$= 8.0 Hz, $J_2$= 18.0 Hz, 1H), 2.25 (s, 3H), 2.20 (s, 3H), 2.00 (s, 3H), 1.95-1.90 (m, 3H), 0.87 (d, *J*=6.6 Hz, 6H), 0.76 (d, *J*=6.0 Hz, 3H).

ESI-MS m/z: Calcd. for $C_{36}H_{45}N_5O_8$: 675.77. Found $(M+H)^+$: 676.3.

Example 46




43 52

To a solution of **43** (33 mg, 0.0557 ml) in $CH_2Cl_2$ (0.4 ml), trans-3-trifluoromethyl cinnamoyl chloride (9.52 ml, 0.0557 ml) and pyridine (4.5 ml, 0.0557 ml) were added at 0 °C. The reaction mixture was stirred for 1h and then, the solution was diluted with $CH_2Cl_2$ (5 ml) and washed with 0.1 N HCl (3 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography ($SiO_2$, Hex: ethyl acetate 1:2) to afford **52** (40 mg, 92%) as a white solid.

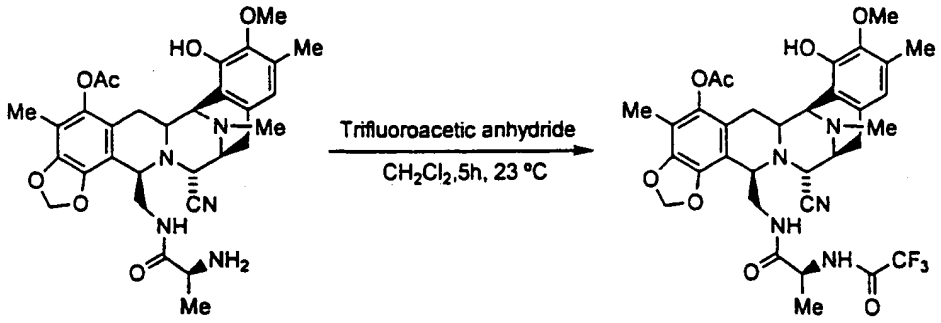
Rf: 0.21 (hexane:ethyl acetate 1:2).

$^1$H NMR (300 MHz, $CD_3OD$). δ 7.74-7.47 (m, 4H), 6.49 (s, 1H), 6.40 (d, *J*= 15.6 Hz, 1H), 6.00 (d, *J*= 1.5 Hz, 1H), 5.90 (d, *J*= 1.5 Hz, 1H), 5.47 (t, *J*= 6 Hz, 1H), 4.12-4.09 (m, 3H), 3.93 (bs, 1H), 3.71 (s, 3H), 3.59-3.58 (m, 1H), 3.38 (d, *J*=7.8 Hz, 1H), 3.29 (d, *J*=12.0 Hz, 1H), 3.00 (dd, $J_1$= 8.1 Hz, $J_2$= 18.3 Hz, 1H), 2.79-2.78 (m, 1H), 2.65 (d, *J*=18.3 Hz, 1H) 2.29 (s, 6H), 2.28 (s, 3H), 2.22 (s, 3H), 1.84-1.80 (m, 1H), 0.85-0.84 (m, 3H).

$^{13}$C NMR (75 MHz, $CDCl_3$) δ 171.9, 168.8, 164.4, 146.9, 144.6, 143.0, 140.5, 140.5, 139.3, 135.7, 131.1, 131.0, 129.4, 129.1, 126.0, 124.1, 124.0, 122.4, 121.1, 120.7, 120.6, 117.7, 116.9, 112.8, 112.0, 101.6, 60.6, 59.3, 57.1, 56.3, 55.9, 55.2, 49.0, 41.7, 49.9, 26.5, 25.1, 20.2, 18.4, 15.7, 9.3.

ESI-MS m/z: Calcd. for $C_{41}H_{42}F_3N_5O_8$: 789.8. Found $(M+H)^+$: 790.3.

Example 47




43 53

To a solution of **43** (10 mg, 0.0169 ml) in $CH_2Cl_2$ (0.2 ml) trifluoroacetic anhydride (2.38µl, 0.0169 ml) was added at 23 °C. The reaction mixture was stirred for 5h and then, the solution was diluted with $CH_2Cl_2$ (5 ml) and washed with 0.1 N HCl (3 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography ($SiO_2$, Hex: ethyl acetate 3:2) to afford **53** (10.7 mg, 93%) as a white solid.

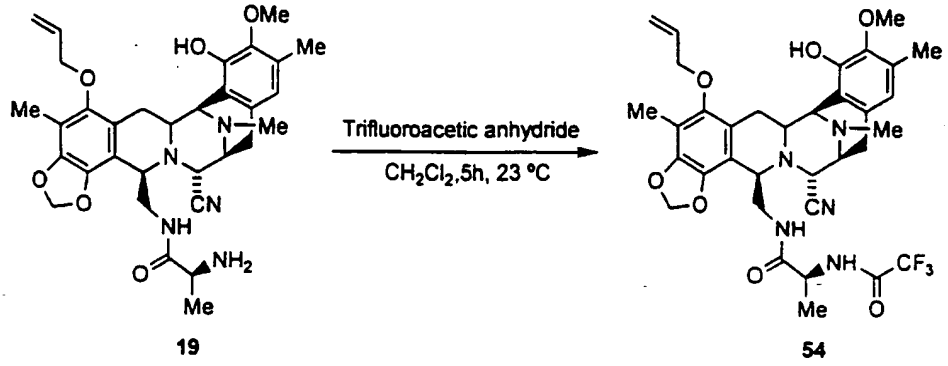
Rf: 0.57 (ethyl acetate:methanol5:1).

$^1$H NMR (300 MHz, $CDCl_3$) δ 6.45 (s, 1H), 6.00 (d, $J$= 1.2 Hz, 1H), 5.90 (d, $J$= 1.2 Hz, 1H), 5.87 (bs, 1H), 5.32 (bs, 1H), 4.12(d, $J$= 2.1 Hz, 1H), 4.08 (d, $J$= 1.8 Hz, 1H), 3.78-3.56 (m, 3H), 3.72 (s, 3H), 3.40 (d, $J$= 8.1 Hz, 1H), 3.25 (d, $J$= 9.3 Hz, 1H), 3.00 (dd, $J_1$= 8.4 Hz, $J_2$= 18.0 Hz, 1H), 2.77 (dd, $J_1$= 2.1 Hz, $J_2$= 15.9 Hz, 1H), 2.68 (d, $J$= 18.6 Hz, 1H), 2.30 (s, 3H), 2.28 (s, 3H), 2.22 (s, 3H), 2.00 (s, 3H), 1.75 (dd, $J_1$= 11.4 Hz, $J_2$= 15.9 Hz, 1H), 0.69 (d, $J$= 6.3 Hz, 3H).

$^{13}$C NMR (75 MHz, $CDCl_3$) δ 170.1, 168.6,156.0, 147.0, 144.6, 143.0, 140.6, 140.4, 131.0, 129.4, 120.9, 120.7, 117.6, 116.8, 112.4, 112.1, 101.6, 60.5, 59.0, 57.1, 56.3, 55.6, 55.2, 48.7, 41.6, 39.4, 26.5, 24.9, 20.2, 17.8, 15.4, 9.2.

ESI-MS m/z: Calcd. for $C_{33}H_{36}F_3N_5O_8$: 687.63. Found $(M+H)^+$: 688.66.

Example 48




19 54

To a solution of **19** (11 mg, 0.0169 ml) in $CH_2Cl_2$ (0.2 ml) trifluoroacetic anhydride (2.38 ml, 0.0169 ml) was added at 23 °C. The reaction mixture was stirred for 5h and then, the solution was diluted with $CH_2Cl_2$ (5 ml) and washed with 0.1 N HCl (3 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography ($SiO_2$, Hex: ethyl acetate 3:2) to afford **54** (10.7 mg, 93%) as a white solid.

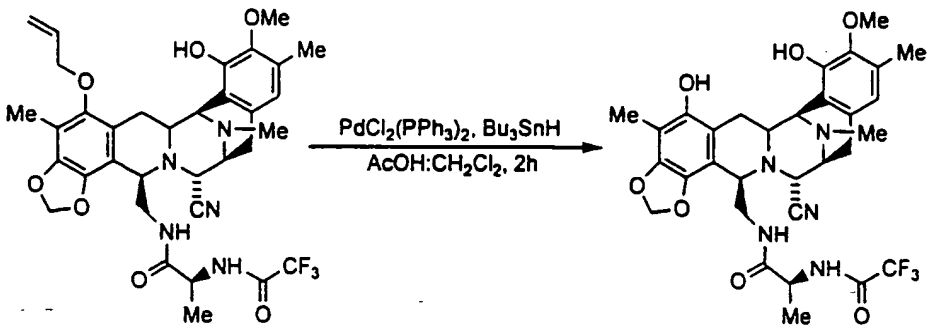
Rf: 0.6 (ethyl acetate:methanol5:1).

$^1$H NMR (300 MHz, $CDCl_3$) δ 7.33 (d, *J*= 6.3 Hz, 1H), 6.45 (s, 1H), 6.04 (m, 1H), 5.95 (d, *J*= 1.5 Hz, 1H), 5.84 (d, *J*= 1.5 Hz, 1H), 5.32 (m, 2 h), 5.21 (m, 1H), 4.11 (m, 4H), 3.73 (s, 3H), 3.64 (m, 2 h), 3.51 (m, 1H), 3.37 (d, *J*= 7.8 Hz, 1H), 3.22 (m, 2 h), 3.03 (dd, 1H, $J_1$= 8.1 Hz, $J_2$= 18.3 Hz, 1H), 2.60 (d, *J*= 18.3 Hz, 1H), 2.29 (s, 3H), 2.24 (s, 3H), 2.08 (s, 3H), 1.86 (dd, $J_1$= 12 Hz, $J_2$= 16.2 Hz, 1H), 0.82 (d, *J*= 7.2 Hz, 3H).

$^{13}$C NMR (75 MHz, $CDCl_3$) δ 170.0, 156.0, 148.4, 147.1, 144.3, 143.0, 138.7, 133.8, 130.5, 129.4, 120.6, 120.4, 117.6, 117.5, 117.0, 113.5, 112.5, 112.4, 101.1, 74.1, 66.8, 60.4, 59.3, 56.9, 56.6, 56.3, 55.4, 48.7, 41.6, 40.1, 26.2, 25.0, 17.6, 15.4, 9.1.

ESI-MS m/z: Calcd. for $C_{35}H_{39}F_3N_5O_7$: 685.69. Found $(M+H)^+$: 686.3.

Example 49




54 55

To a solution of **54** (100 mg, 0.415 ml) in $CH_2Cl_2$ (4 ml), acetic acid (40 ml), $(PPh_3)_2PdCl_2$ (8.4 mg, 0.012 ml) and $Bu_3SnH$ (157 ml, 0.56 ml) were added at 23 °C. After stirring at that temperature for 2 h the reaction was poured into a pad of flash column ($SiO_2$, gradient Hex to hexane:ethyl acetate 2:1) to afford **55** (90 mg, 96%) as a white solid.

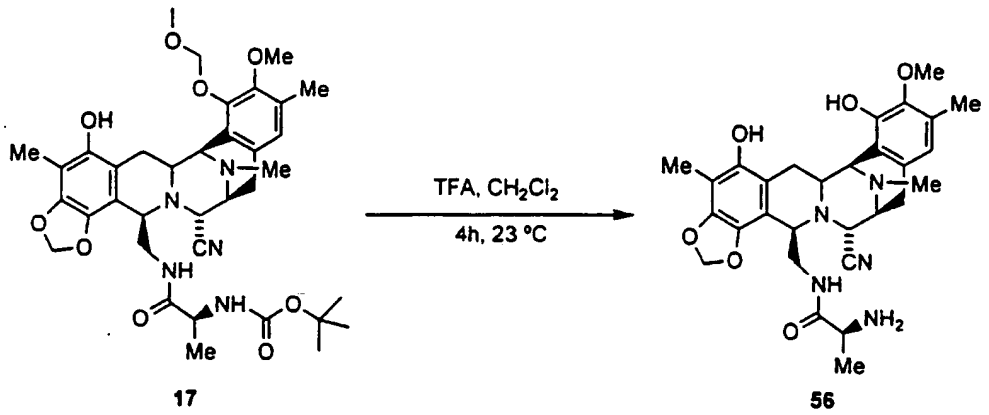
Rf: 0.6 (hexane:ethyl acetate 1:2).

$^1$H NMR (300 MHz, $CDCl_3$) δ 7.55 (d, *J*= 7.2 Hz, 1H), 6.45 (s, 1H), 5.90 (d, *J*= 1.2 Hz, 1H), 5.82 (d, *J*= 1.2 Hz, 1H), 5.37 (t, *J*= 6.0 Hz, 1H), 4.15 (d, *J*= 2.1 Hz, 1H), 4.04 (d, *J*= 1.8 Hz, 1H), 3.70 (s, 3H), 3.66-3.53 (m, 2 h), 3.37-3.31 (m, 2 h), 3.19-3.15 (d, *J*= 11.7 Hz, 1H), 3.08-3.00 (m, 2 h), 2.56 (d, *J*=18.3 Hz, 1H), 2.30 (s, 3H), 2.24 (s, 3H), 2.04 (s, 3H), 1.91 (dd, $J_1$= 12.0 Hz, $J_2$= 15.6 Hz, 1H), 0.84 (d, *J*= 6.9 Hz, 3H).

$^{13}$C NMR (75 MHz, $CDCl_3$) δ 170.1, 156.3, 147.3, 144.9, 144.4, 143.3, 136.7, 130.7, 129.3, 120.6, 117.6, 117.4, 114.4, 112.1, 107.7, 101.0, 85.8, 60.5, 59.3, 56.5, 56.4, 56.2, 55.2, 48.9, 41.6, 40.9, 25.7, 25.3, 18.0, 15.6, 8.7.

ESI-MS m/z: Calcd. for $C_{32\,h35}F_3N_5O_7$: 645.63. Found $(M+H)^+$: 646.2.

Example 50




17 56

To a solution of **17** (200 mg, 0.288 ml) in $CH_2Cl_2$ (1.44 ml), trifluoroacetic acid (888 ml, 11.53 ml) was added and the reaction mixture was stirred for 4h at 23 °C. The reaction was quenched at 0 °C with saturated aqueous sodium bicarbonate (60 ml) and extracted with ethyl acetate (2 x 70 ml). The combined organic layers were dried (sodium sulphate) and concentrated *in vacuo* to afford **56** (147 mg, 93%) as a white solid that was used in subsequent reactions with no further purification.

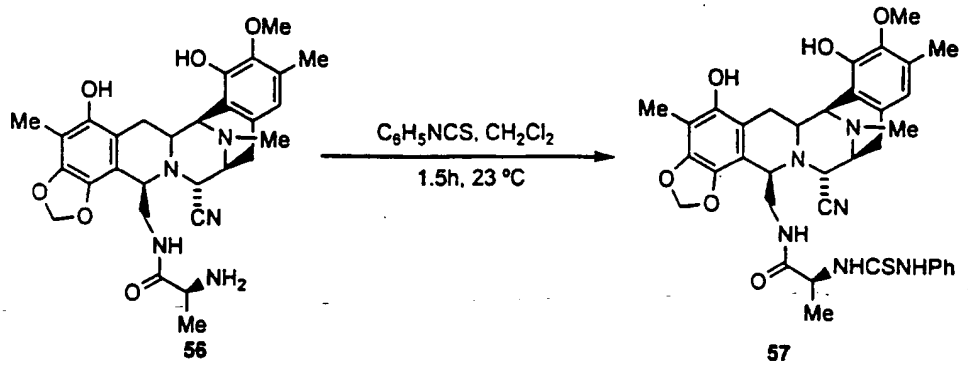
Rf: 0.19 (ethyl acetate:methanol5:1).

$^1$H NMR (300 MHz, $CD_3OD$). δ 6.48 (s, 1H), 5.88, d, *J*= 0.9 Hz, 1H), 5.81 (d, *J*= 0.9 Hz, 1H), 4.35 (d, *J*= 2.4 Hz, 1H),4.15 (d, *J*= 1.8 Hz, 1H), 3.99-3.98 (m, 1H), 3.70 (s, 3H), 3.52-2.96 (m, 7H), 2.68 (d, *J*= 18.3 Hz, 1H), 2.24 (s, 3H), 2.23 (s, 3H), 2.06 (s, 3H), 1.85 (dd, $J_1$= 11.7 Hz, $J_2$= 15.6 Hz, 1H), 0.91 (d, *J*= 6.6 Hz, 3H).

$^{13}$C NMR (75 MHz, $CD_3OD$): δ 173.2, 149.1, 145.6, 144.9, 138.0, 132.2, 130.6, 121.4, 119.6, 117.4, 114.3, 109.2, 102.5, 82.3, 60.4, 58.4, 58.3, 57.8, 56.6, 50.1, 42.3, 41.6, 27.8, 26.2, 19.5, 15.5, 9.8.

ESI-MS m/z: Calcd. for $C_{29}H_{35}N_5O_6$: 549.62. Found $(M+H)^+$: 550.3.

Example 51




To a solution of **56** (10 mg, 0.018 ml) in $CH_2Cl_2$ (0.4 ml), phenyl isothiocyanate (13 ml, 0.109 ml) was added and the reaction was stirred at 23° C for 1.5h. The mixture was concentrated *in vacuo* and the residue was purified by flash column chromatography ($SiO_2$, gradient Hexane to 1:1 hexane:ethyl acetate) to afford **57** (8 mg, 65%) as a white solid.

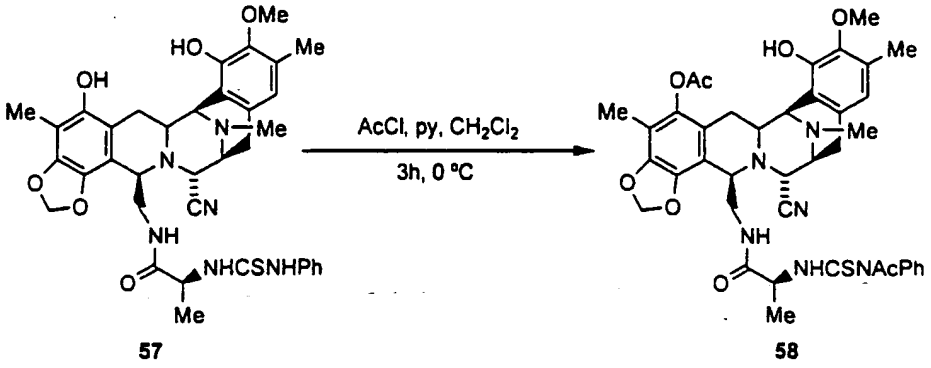
Rf: 0.57 (ethyl acetate:methanol10:1).

$^1$H NMR (300 MHz, $CDCl_3$): δ 7.88 (bs, 1H), 7.41-7.36 (m, 2 h), 7.27-7.22 (m, 1H), 7.02-7.00 (d, *J*= 7.8 Hz, 2 h), 6.71 (d, *J*= 7.2 Hz, 1H), 6.31 (s, 1H), 6.17 (bs, 1H), 5.93 (d, *J*=1.2 Hz, 1H), 5.83 (d, *J*= 1.2 Hz, 1H), 5.55 (bs, 1H), 5.20-5.17 (m, 1H), 4.16 (d, *J*= 1.8 Hz, 1H), 4.05 (bs, 1H), 4.02 (d, *J*= 2.4 Hz, 1H), 3.79 (s, 3H), 3.75-3.71 (m, 1H), 3.35 (d, *J*= 7.8 Hz, 1H), 3.28-3.19 (m, 2 h), 3.12-2.97 (m, 2 h), 2.50 (d, *J*=18.3 Hz, 1H), 2.32 (s, 3H), 2.21 (s, 3H), 2.15-2.09 (dd, $J_1$= 11.4 Hz, $J_2$= 15.9 Hz, 1H), 1.95 (s, 3H), 0.88 (d, *J*=6.9 Hz, 3H).

$^{13}$C NMR (75 MHz, $CDCl_3$): δ 178.5, 171.7, 147.2, 145.0, 144.3, 143.3, 137.0, 135.7, 130.6, 130.4, 129.6, 127.5, 124.3, 120.6, 117.7, 117.2, 115.3, 112.1, 108.3, 100.9, 60.9, 59.5, 56.7, 56.5, 56.2, 55.2, 54.1, 41.7, 41.1, 26.3, 25.4, 18.5, 15.8, 9.0.

ESI-MS m/z: Calcd. for $C_{36}H_{40}N_6O_6S$: 684.81. Found $(M+H)^+$: 685.3.

Example 52




57 58

To a solution of **57** (45 mg, 0.065 ml) in $CH_2Cl_2$ (0.5 ml), acetyl chloride (4.67 ml, 0.065 ml) and pyridine (5.3 ml, 0.065 ml) were added at 0 °C. The reaction mixture was stirred for 3h and then, the solution was diluted with $CH_2Cl_2$ (10 ml) and washed with 0.1 N HCl (5 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography (RP-18, $CH_3CN$: $H_2O$ 40:60) to afford **58** (14 mg, 28%) as a white solid.

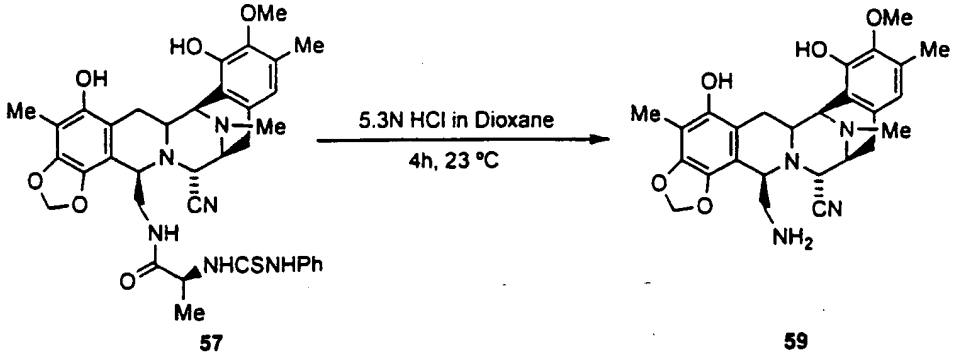
Rf: 0.34 ($CH_3CN$: $H_2O$ 7:15).

$^1H$ NMR (300 MHz, $CDCl_3$). δ 11.90 (d, *J*= 6.6 Hz, 1H), 7.45-7.40 (m, 3H), 7.18-7.15 (m, 2 h), 6.58 (s, 1H), 6.00 (d, *J*= 1.2 Hz, 1H), 5.89 (d, *J*= 1.2 Hz, 1H), 5.70 (s, 1H), 5.37 (t, *J*= 4.8 Hz, 1H), 4.48 (m, 1H), 4.23 (bs, 1H), 4.07 (bs, 2 h), 3.85-3.75 (m, 1H), 3.70 (s, 3H), 3.46-3.41 (m, 2 h), 3.24-3.20 (m, 1H), 3.00-2.95 (m, 1H), 2.87-2.75 (m, 1H), 2.31 (s, 3H), 2.28 (s, 3H), 2.24 (s, 3H), 2.00 (s, 3H), 1.85 (dd, $J_1$= 11.4 Hz, $J_2$= 15.6 Hz, 1H), 1.66 (s, 3H), 0.82 (d, *J*= 6.0 Hz, 3H).

$^{13}C$ NMR (75 MHz, $CDCl_3$) ): δ 182.6, 174.3, 171.0, 146.6, 144.6, 142.7, 142.3, 140.7, 140.2, 131.3, 129.8, 129.3, 128.9, 128.8, 121.5, 120.4, 117.3, 116.6, 112.8, 112.0, 111.3, 101.5, 60.5, 59.0, 57.6, 56.2, 55.9, 55.3, 55.1, 41.6, 39.4, 27.8, 26.5, 24.8, 20.2, 17.1, 15.5, 9.3.

ESI-MS m/z: Calcd. for $C_{40}H_{44}N_6O_8S$: 768.88. Found $(M+H)^+$: 769.2.

Example 53




57 59

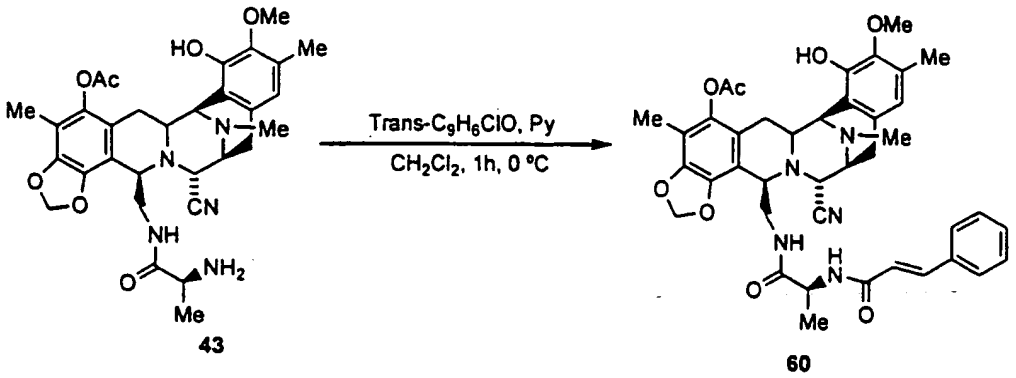
A solution of **57** (130 mg, 0.189 ml) in dioxane (1 ml), 5.3N HCl/dioxane (1.87 ml) was added and the reaction was stirred at 23 °C for 4h. Then, $CH_2Cl_2$ (15 ml) and $H_2O$ (10 ml) were added to this reaction and the organic layer was decanted. The aqueous phase was basified with saturated aq sodium bicarbonate (60 ml) (pH = 8) at 0 °C and then, extracted with ethyl acetate (2x50 ml). The combined organic extracts were dried (sodium sulphate), and concentrated *in vacuo* to afford **59** (63 mg, 70%) as a white solid.

Rf: 0.15 (ethyl acetate:methanol5:1).

$^1$H NMR (300 MHz, $CDCl_3$). δ 6.67 (s, 1H), 5.99 (d, $J$= 0.9 Hz, 1H), 5.91 (d, $J$= 1.2 Hz, 1H), 5.10 (bs, 1H), 4.32 (d, $J$= 7.2 Hz, 1H), 4.25 (dd, $J_1$= 3.6 Hz, $J_2$= 9.3 Hz, 1H), 3.7 (s, 3H), 3.71-3.64 (m, 2 h), 3.50 (dd, $J_1$= 2.4 Hz, $J_2$= 15.9 Hz, 1H), 3.42-3.37 (m, 2 h), 3.16 (dd, $J_1$=3.6 Hz, $J_2$= 12.9 Hz, 1H), 2.57 (dd, $J_1$= 9.3 Hz, $J_2$= 12.9 Hz, 1H), 2.27 (s, 3H), 2.11 (s, 3H), 1.91 (dd, $J_1$= 12.0 Hz, $J_2$= 15.9 Hz, 1H).

ESI-MS m/z: Calcd. for $C_{26}H_{30}N_4O_5$: 478.5. Found $(M+H)^+$: 479.3.

Example 54




43 60

A solution of **43** (20 mg, 0.0338 mmol) in $CH_2Cl_2$ (0.3 ml), cinnamoyl chloride (5.63 mg , 0.0338 mmol) and pyridine (2.73 ml, 0.0338 mmol) were added at 0 °C. The reaction mixture was stirred for 1h and then, the solution was diluted with $CH_2Cl_2$ (10 ml) and washed with 0.1 N HCl (5 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography ($SiO_2$, EtOAc:MeOH 20:1) to afford **60** (22 mg, 90%) as a white solid.

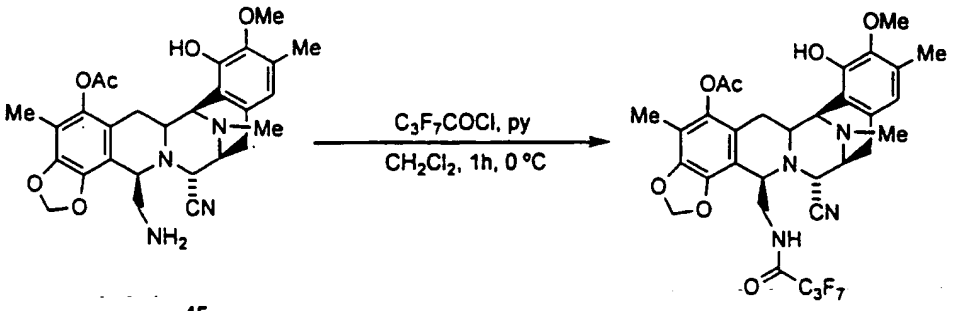
Rf: 0.56 (EtOAc:MeOH 5:1).

$^1$H NMR (300 MHz, $CDCl_3$). 7.51 (s, 1H), 7.50-7.47 (m, 2H), 7.36-7.35 (m, 2H), 6.43 (s, 1H), 6.36 (brd, *J*= 15.9 Hz, 2H), 6.01 (d, *J*= 1.5 Hz, 1H), 5.90 (brd, *J*= 1.5 Hz, 2H), 5.42 (t, *J*= 6.0 Hz 1H), 4.12-4.07 (m, 3H), 3.96-3.95 (m, 1H), 3.73 (bs, 3H), 3.58 (bs, 2H), 3.39 (d, *J*= 8.7 Hz, 1H), 3.25 (d, *J*= 11.7 Hz, 1H), 3.0 (dd, $J_1$= 7.5 Hz, $J_2$= 17.7 Hz, 1H), 2.78 (d, *J*= 15.9 Hz, 1H), 2.67 (d, *J*= 16.5 Hz, 1H), 2.29 (s, 6H), 2.23 (s, 3H), 1.99 (s, 3H), 1.82 (dd, $J_1$= 11.4 Hz, $J_2$= 15.6 Hz, 1H), 0.83 (d, *J*= 6.0 Hz, 3H).

$^{13}$C NMR (75 MHz, $CDCl_3$) ): δ. 172.0, 165.0, 146.9, 144.6, 143.1, 141.0, 140.5, 134.8, 131.0, 129.7, 129.1, 128.8, 127.8, 125.5, 123.8, 123.0, 121.1, 120.5, 117.7, 116.9, 112.8, 112.0, 101.9, 60.6, 59.2, 57.1, 56.4, 55.9, 55.3, 48.8, 41.7, 40.0, 26.5, 25.1, 20.3, 18.5, 15.7, 9.3.

ESI-MS m/z: Calcd. for $C_{40}H_{43}N_5O_8$: 721.8. Found $(M+H)^+$: 722.3.

Example 55




45 61

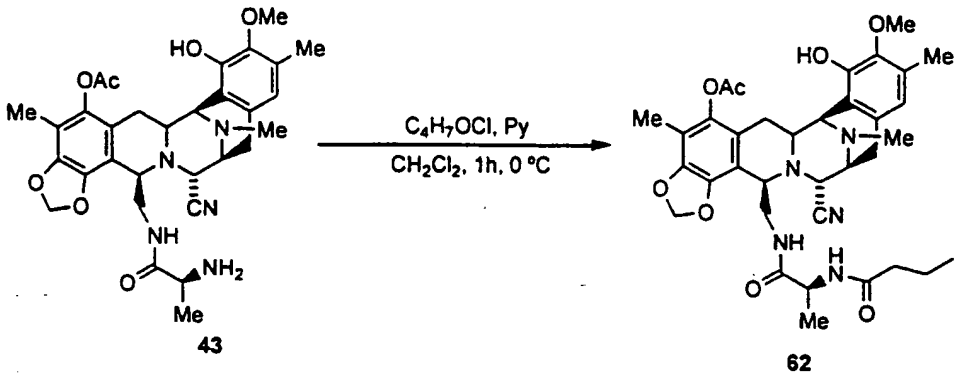
A solution of **45** (19 mg, 0.0364 mmol) in $CH_2Cl_2$ (0.3 ml), heptafluorobutyryl chloride ( 5.44 ml, 0.0364 mmol) and pyridine (2.95 ml, 0.0364 mmol) were added at 0 °C. The reaction mixture was stirred for 1h and then, the solution was diluted with $CH_2Cl_2$ (10 ml) and washed with 0.1 N HCl (5 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography ($SiO_2$, EtOAc:MeOH 20:1) to afford **61** (11.7 mg, 45%) as a white solid.

Rf: 0.76 (EtOAc:MeOH 5:1).

$^1$H NMR (300 MHz, $CDCl_3$) δ 6.46 (s, 1H), 6.12 (bs, 1H), 5.98 (d, *J*= 1.2 Hz, 1H), 5.93 (d, *J*= 1.2 Hz, 1H), 5.72 (bs, 1H), 4.13-4.11 (m, 2H), 4.0 (d, *J*= 2.4 Hz, 1H), 3.98-3.96 (m, 1H), 3.73 (s, 3H), 3.39 (d, *J*= 7.5 Hz, 1H), 3.39-3.28 (m, 2H), 3.09 (dd, $J_1$= 8.1 Hz, $J_2$= 18.0 Hz, 1H), 2.80 (d, *J*= 16.2 Hz, 1H), 2.46 (d, *J*= 18.3 Hz, 1H), 2.32 (s, 6H), 2.21 (s, 3H), 1.99 (s, 3H), 1.80 (dd, $J_1$= 12.0 Hz, $J_2$= 16.2 Hz, 1H).

ESI-MS m/z: Calcd. for $C_{32}H_{31}F_7N_4O_7$: 716.6. Found $(M+H)^+$: 717.2.

Example 56




43 62

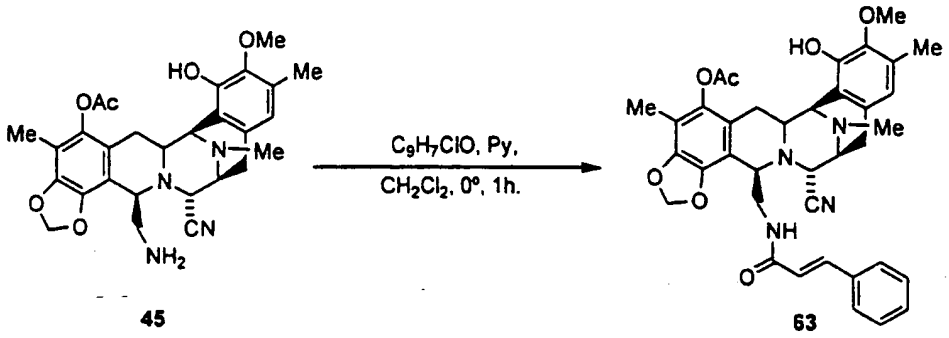
A solution of **43** (24 mg, 0.04 mmol) in $CH_2Cl_2$ (0.3 ml), butyryl chloride (4.15 ml, 0.04 mmol) and pyridine (3.28 ml, 0.04 mmol) were added at 0 °C. The reaction mixture was stirred for 1h and then, the solution was diluted with $CH_2Cl_2$ (10 ml) and washed with 0.1 N HCl (5 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography ($SiO_2$, EtOAc:MeOH 20:1) to afford **62** (24 mg, 90%) as a white solid.

Rf: 0.35 (EtOAc:MeOH 5:1).

$^1$H NMR (300 MHz, $CDCl_3$) δ 6.47 (s, 1H), 6.10 (d, *J*= 6.5 Hz, 1H), 6.0 (d, *J*= 1.5 Hz, 1H), 5.91 (d, *J*= 1.5 Hz, 1H), 5.86 (bs, 1H), 5.31 (d, *J*= 6.9 Hz, 1H), 4.11-4.06 (m, 3H), 3.85-3.81 (m, 1H), 3.75 (s, 3H), 3.59-3.53 (m, 2H), 3.38 (d, *J*= 7.5 Hz, 1H), 3.27-3.22 (m, 1H), 3.0 (dd, $J_1$= 7.8 Hz, $J_2$= 17.4 Hz, 1H), 2.79 (d, *J*= 15.3 Hz, 1H), 2.63 (d, *J*= 17.7 Hz, 1H), 2.31 (s, 3H), 2.0 (s, 3H), 1.80 (dd, $J_1$= 12.0 Hz, $J_2$= 15.9 Hz, 1H), 1.58 (q, *J*= 7.2 Hz, 2H), 0.89 (t, *J*= 7.2 Hz, 3H), 0.76 (d, *J*= 6.6 Hz, 3H).

ESI-MS m/z: Calcd. for $C_{35}H_{43}N_5O_8$: 661.64. Found $(M+H)^+$: 662.3

Example 57




45 63

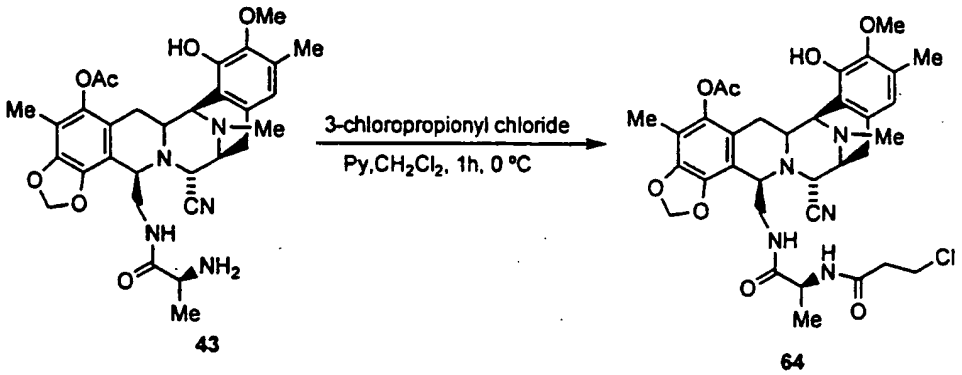
A solution of **43** (19 mg, 0.0364 mmol) in $CH_2Cl_2$ (0.3 ml), cinnamoyl chloride (6.06 mg , 0.0364 mmol) and pyridine (2.95 ml, 0.0364 mmol) were added at 0 °C. The reaction mixture was stirred for 1h and then, the solution was diluted with $CH_2Cl_2$ (10 ml) and washed with 0.1 N HCl (5 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography ($SiO_2$, EtOAc:MeOH 20:1) to afford **63** (20.1 mg, 85%) as a white solid.

Rf: 0.65 (EtOAc:MeOH 5:1).

$^1$H NMR (300 MHz, $CDCl_3$) δ 7.39-7.29 (m, 5H), 6.42, (s, 1H), 6.01 (d, *J*= 1.5 Hz, 1H), 5.92 (d, *J*= 1.5 Hz, 1H), 5.73 (bs, 1H), 5.24 (t, *J*= 6.8 Hz, 1H), 4.12-4.08 (m, 3H), 3.66-3.64 (m, 2H), 3.58 (bs, 3H), 3.36 (d, *J*= 8.7 Hz, 1H), 3.29 (d, *J*= 12.0 Hz, 1H), 2.98 (dd, $J_1$= 8.1 Hz, $J_2$= 18 Hz, 1H), 2.33 (s, 6H), 2.29 (s, 3H), 2.01 (s, 3H), 1.84 (dd, $J_1$= 12.0 Hz, $J_2$= 15.9 Hz, 1H). ).

ESI-MS m/z: Calcd. for $C_{37}H_{38}N_4O_7$: 650.72. Found $(M+H)^+$: 651.2.

Example 58



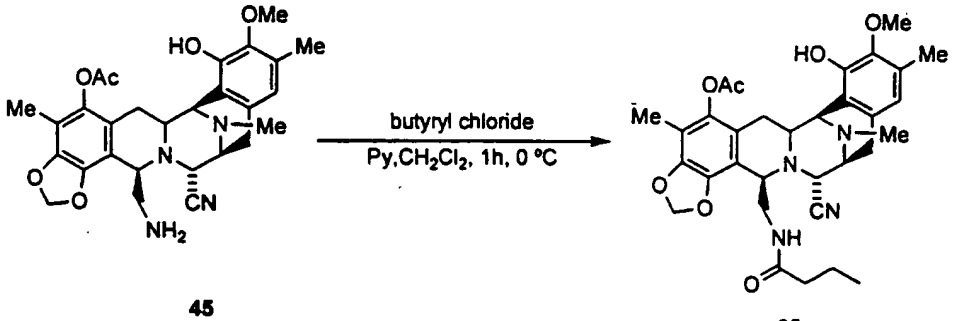

43 64

A solution of **43** (20 mg, 0.0338 mmol) in $CH_2Cl_2$ (0.3 ml), 3-chloropropionyl chloride (3.22 ml , 0.0338 mmol) and pyridine (2.73 ml, 0.0338 mmol) were added at 0 °C. The reaction mixture was stirred for 1h and then, the solution was diluted with $CH_2Cl_2$ (10 ml) and washed with 0.1 N HCl (5 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography ($SiO_2$, EtOAc:MeOH 20:1) to afford **64** (20.5 mg, 89%) as a white solid.

Rf: 0.32 (EtOAc:Hexane 5:1).

$^1$H NMR (300 MHz, $CDCl_3$) 6.48 (s, 3H), 6.28 (m, 1H), 5.99 (d, *J*= 1.2 Hz, 1H), 5.91 (d, *J*= 1.2 Hz, 1H), 5.86 (bs, 1H), 5.31 (m, 1H), 4.08-4.07 (m, 3H), 3.75 (s, 3H), 3.72-3.53 (m, 5H), 3.39 (d, *J*= 8.1 Hz, 1H), 3.24 (d, *J*= 12.0 Hz, 1H), 3.00 (dd, $J_1$= 8.1 Hz, $J_2$= 18.0 Hz, 1H), 2.79 (d, *J*= 13.5 Hz, 1H), 2.50 (t, *J*= 6.3 Hz, 2H), 2.32 (s, 3H), 2.28 (s, 3H), 2.25 (s, 3H), 2.0 (s, 3H), 1.79 (dd, $J_1$= 12.3 Hz, $J_2$= 14.8 Hz, 1H), 0.81 (d, *J*= 6.3 Hz, 3H).

Example 59




**45** **65**

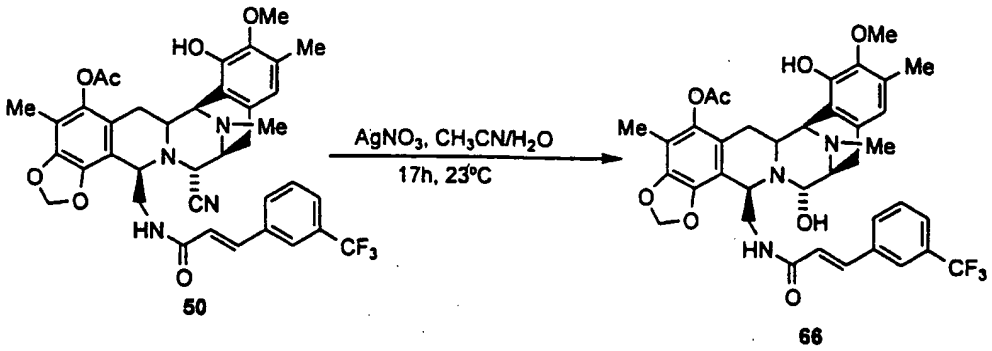
A solution of **43** (19 mg, 0.0364 mmol) in $CH_2Cl_2$ (0.3 ml), butyryl chloride (3.78 ml , 0.0364 mmol) and pyridine (2.95 ml, 0.0364 mmol) were added at 0 °C. The reaction mixture was stirred for 1h and then, the solution was diluted with $CH_2Cl_2$ (10 ml) and washed with 0.1 N HCl (5 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography ($SiO_2$, EtOAc:MeOH 20:1) to afford **64** (19 mg, 87%) as a white solid.

Rf: 0.60 (EtOAc:MeOH 5:1).

$^1$H NMR (300 MHz, $CDCl_3$) 6.50 (s, 1H), 5.98 (d, $J$= 1.5 Hz, 1H), 5.91 (d, $J$= 1.5 Hz, 1H), 5.75 (s,1H), 5.01 (t, $J$= 6.4 Hz, 1H), 4.10 –4.09 (m, 1H), 4.06 (d, $J$= 2.1 Hz, 1H), 4.03-4.02 (m, 1H), 3.76 (s, 3H), 3.67-3.60 (m, 1H), 3.42-3.35 (m, 2H), 3.29 (d, $J$= 12.0 Hz, 1H), 3.02 (dd, $J_1$= 7.8 Hz, $J_2$= 17.7 Hz, 1H), 2.79 (d, $J$= 14.1 Hz, 1H), 2.56 (d, $J$= 18.3 Hz, 1H), 2.32 (s, 3H), 2.31 (s, 3H), 2.25 (s, 3H), 1.78 (dd, $J_1$= 12.0 Hz, $J_2$= 15.9 Hz, 1H), 1.63 (s, 3H), 1.53-1.46 (m, 2H), 1.28-1.16 (m, 2H), 0.68 (t, $J$= 7.2 Hz, 3H).

ESI-MS m/z: Calcd. for $C_{32}H_{38}N_4O_7$: 590.67. Found $(M+H)^+$: 591.2.

Example 60



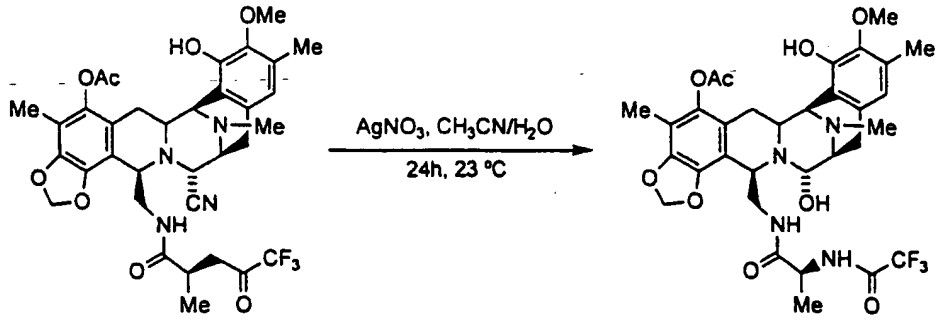

To a solution of **50** (31.7 mg, 0.044 mmol) in $CH_3CN/H_2O$ (1.5 ml/0.5 ml), $AgNO_3$ (225 mg, 1.32 mmol) was added and the reaction was stirred at 23°C for 17 h. Then brine (10 ml) and Aq sat $NaHCO_3$ (10 ml) were added at 0°C and the mixture was stirred for 15 min, filtered through a pad of celite and washed with $CH_2Cl_2$ (20 ml). The solution was decanted and the organic layer was dried and concentrated *in vacuo*. The residue was purified by flash column chromatography ($SiO_2$, EtOAc:MeOH 5:1) to afford **66** (16 mg, 51%) as a white solid.

Rf: 0.26 (EtOAc:MeOH 5:1).

$^1$H NMR (300 MHz, $CDCl_3$) δ 7.66-7.42 (m, 4H), 7.20 (bs, 1H), 6.44 (s, 1H), 5.97 (b, $J$= 1.2 Hz, 1H), 5.90 (d, $J$= 1.2 Hz, 1H), 5.76 (bs, 1H), 5.28 (bs, 1H), 4.54 (bs, 1H), 4.43 (bs, 1H), 4.00 (bs, 1H), 3.68-3.57 (m, 4H), 3.47 (d, $J$= 3.3 Hz, 1H), 3.40 (d, $J$= 11.7 Hz, 1H), 3.17 (d, $J$= 6.9 Hz, 1H), 2.92 (dd, $J_1$= 8.1 Hz, $J_2$= 17.7 Hz, 1H), 2.74 (d, $J$= 17.1 Hz, 1H), 2.48 (d, $J$= 18.6 Hz, 1H), 2.32 (s, 6H), 2.28 (s, 3H), 1.99 (s, 3H), 1.76 (dd, $J_1$= 12.0 Hz, $J_2$= 16.2 Hz, 1H).

ESI-MS m/z: Calcd. for $C_{37}H_{38}F_3N_3O_8$: 709. Found ($M^+$-17): 692.3.

Example 61




53 67

To a solution of **53** (57 mg, 0.0828 mmol) in $CH_3CN/H_2O$ (1.5 mL/0.5 ml), $AgNO_3$ (650 mg, 3.81 mmol) was added and the reaction was stirred at 23°C for 24 h. Then, brine (10 ml) and Aq sat $NaHCO_3$ (10 ml) were added at 0°C and the mixture was stirred for 15 min, filtered through a pad of celite and washed with $CH_2Cl_2$ (20 ml). The solution was decanted and the organic layer was dried and concentrated *in vacuo*. The residue was purified by flash column chromatography ($SiO_2$, EtOAc:MeOH 5:1) to afford **67** (28 mg, 50%) as a white solid.

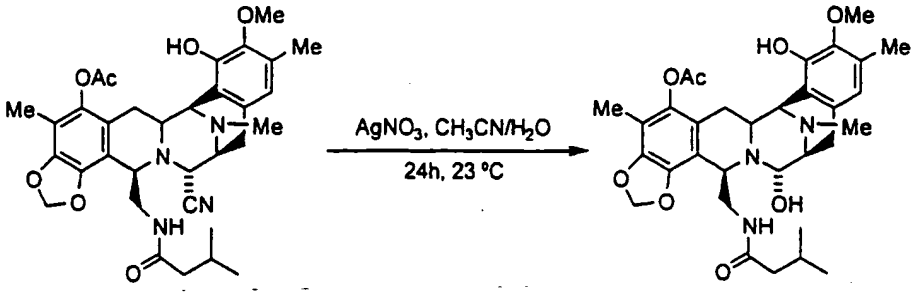
Rf: 0.28 (EtOAc:MeOH 10:1).

$^1H$ NMR (300 MHz, $CDCl_3$) d

6.47 (s, 1H), 5.97 (s, 1H), 5.88 (s, 1H), 5.35 (bs, 1H), 4.51 (bs, 1H), 4.41 (bs, 1H), 4.12-4.05 (m, 1H), 4.00 (d, *J*= 2.7 Hz, 1H), 3.77 (s, 3H), 3.64 (bs, 1H), 3.46 (d, *J*= 3.3 Hz, 1H), 3.34 (d, *J*= 11.4 Hz, 1H), 3.18 (d, *J*= 7.5 Hz, 1H), 2.95 (dd, $J_1$= 8.4 Hz, $J_2$= 18.3 Hz, 1H), 2.70 (d, *J*= 15.6 Hz, 1H), 2.48 (d, *J*= 17.7 Hz, 1H), 2.28 (s, 3H), 2.27 (s, 3H), 2.26 (s, 3H), 1.98 (s, 3H), 1.68 (dd, $J_1$= 12 Hz, $J_2$= 15.6 Hz, 1H), 0.86 (d, *J*= 6.3 Hz, 3H).

ESI-MS m/z: Calcd. for $C_{32}H_{37}F_3N_4O_9$: 678.66. Found ($M^+$-17): 661.2.

Example 62



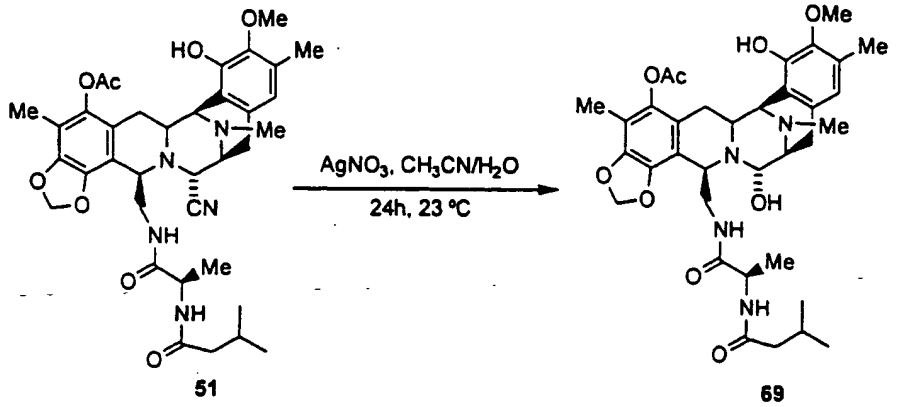

48 68

To a solution of **48** (32 mg, 0.0529 mmol) in $CH_3CN/H_2O$ (1.5 ml/0.5 ml), $AgNO_3$ (270 mg, 1.58 mmol) was added and the reaction was stirred at 23°C for 24 h. Then, brine (10 ml) and Aq sat $NaHCO_3$ (10 ml) were added at 0°C and the mixture was stirred for 15 min, filtered through a pad of celite and washed with $CH_2Cl_2$ (20 ml). The solution was decanted and the organic layer was dried and concentrated *in vacuo*. The residue was purified by flash column chromatography ($SiO_2$, EtOAc:MeOH 5:1) to afford **68** (18 mg, 56%) as a white solid.

Rf: 0.40 (EtOAc:MeOH 5:1).

$^1H$ NMR (300 MHz, $CDCl_3$) d 6.50 (s, 1H), 5.95 (d, *J*= 1.2 Hz, 1H), 5.88 (d, *J*= 1.2 Hz, 1H), 5.23 (d, *J*= 6.9 Hz, 1H), 4.45 (d, *J*= 3.3 Hz, 1H), 4.38 (s, 1H), 4.01 (d, *J*= 2.4 Hz, 1H), 3.78 (m, 1H), 3.77 (s, 3H), 3.41-3.37 (m, 1H), 3.17-3.15 (m, 1H), 2.96 (dd, $J_1$= 7.8 Hz, $J_2$= 18.0 Hz, 1H), 2.70 (d, *J*= 15.3 Hz, 1H), 2.40 (d, *J*= 18.0 Hz, 1H), 2.30 (s, 6H), 2.27 (s, 3H), 1.76-1.65 (m, 1H), 1.35-1.25 (m, 2H), 0.89-0.82 (m, 1H), 0.69 (d, *J*= 6.6 Hz, 3H), 0.58 (d, *J*= 6.6 Hz, 3H)

Example 63



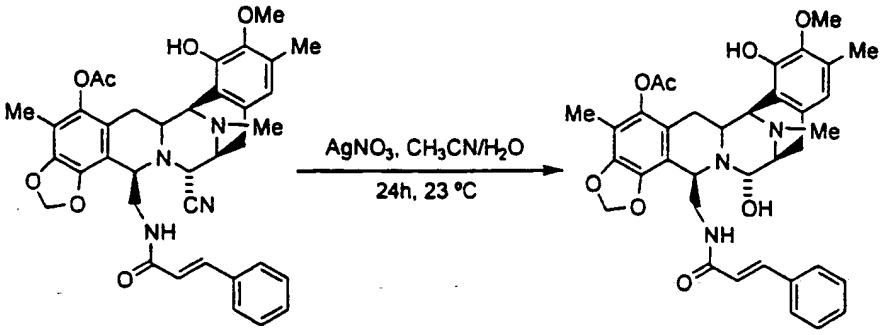

51 69

To a solution of **51** (27 mg, 0.04 mmol) in $CH_3CN/H_2O$ (1.5 ml/0.5 ml), $AgNO_3$ (204 mg, 1.19 mmol) was added and the reaction was stirred at 23°C for 24 h. Then, brine (10 ml) and Aq sat $NaHCO_3$ (10 ml) were added at 0°C and the mixture was stirred for 15 min, filtered through a pad of celite and washed with $CH_2Cl_2$ (20 ml). The solution was decanted and the organic layer was dried and concentrated *in vacuo*. The residue was purified by flash column chromatography ($SiO_2$, EtOAc:MeOH 5:1) to afford **69** (10 mg, 38%) as a white solid.

Rf: 0.38 (EtOAc:MeOH 5:1).

$^1$H NMR (300 MHz, $CDCl_3$) d 6.48 s, 1H), 6.16 (bs, 1H), 5.98 (d, $J$= 1.5 Hz, 1H), 5.89 (d, $J$= 1.5 Hz, 1H), 5.33 (t, $J$= 6.0 Hz, 1H), 4.50 (m, 1H), 4.40 (m, 1H), 4.11-4.09 (m, 1H), 4.00 (d, $J$= 2.6Hz, 1H), 3.78 (s, 3H), 3.41-3.32 (m, 3H), 3.18 (d, $J$= 8.4 Hz, 1H), 2.94 (dd, $J_1$= 8.4 Hz, $J_2$= 18.3 Hz, 1H), 2.70 (d, $J$= 14.4 Hz, 1H), 4.45 (d, $J$= 18.3 Hz, 1H), 2.31 (s, 3H), 2.28 (s, 3H), 2.27 (s, 3H), 2.04 (s, 3H), 2.00-1.86 (m, 3H), 1.73 (m, 1H), 0.87 (d, $J$= 6.3 Hz, 6H).

Example 64



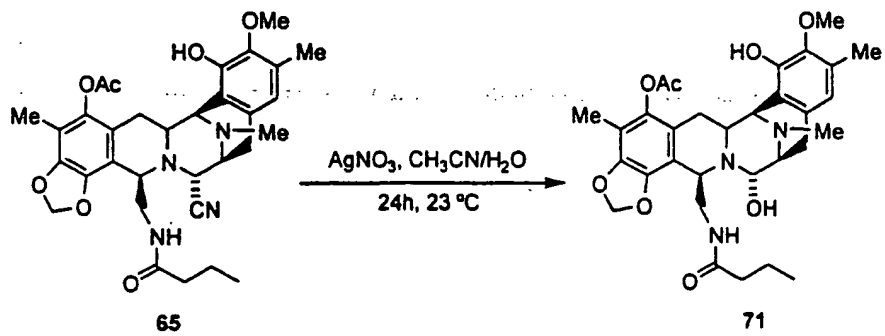

63 70

To a solution of **63** (15 mg, 0.023 mmol) in $CH_3CN/H_2O$ (1.5 ml/0.5 ml), $AgNO_3$ (118 mg, 0.691 mmol) was added and the reaction was stirred at 23°C for 24 h. Then, brine (10 ml) and Aq sat $NaHCO_3$ (10 ml) were added at 0°C and the mixture was stirred for 15 min, filtered through a pad of celite and washed with $CH_2Cl_2$ (20 ml). The solution was decanted and the organic layer was dried and concentrated *in vacuo*. The residue was purified by flash column chromatography ($SiO_2$, EtOAc:MeOH 5:1) to afford **70** (20.1 mg, 85%) as a white solid.

Rf: 0.43 (EtOAc:MeOH 5:1).

$^1$H NMR (300 MHz, $CDCl_3$) d 7.38-7.28 (m, 5H), 6.48 (s, 1H), 5.98 (d, *J*=1.5 Hz, 1H), 5.91 (d, *J*=1.5 Hz, 1H), 5.75 (bs, 1H), 5.38 (brd, 1H), 5.30 (bs, 1H), 4.53 (m, 1H), 4.42 (m, 1H), 4.02 (d, *J*=2.7 Hz, 1H), 3.78-3.65 (m, 5H), 3.46-3.40 (m, 2H), 3.17 (d, *J*=7.8 Hz, 1H), 2.94 (dd, $J_1$=7.8 Hz, $J_2$=17.7 Hz, 1H), 2.73 (d, *J*=16.8 Hz, 1H), 2.45 (d, *J*=18.0 Hz, 1H), 2.31 (s, 6H), 2.28 (s, 3H), 1.97 (s, 3H), 1.77 (dd, $J_1$=12.0 Hz, $J_2$=15.3 Hz, 1H).

Example 65




65 71

To a solution of **65** (25 mg, 0.042 mmol) in $CH_3CN/H_2O$ (1.5 ml/0.5 ml), $AgNO_3$ (215.56 mg, 1.269 mmol) was added and the reaction was stirred at 23°C for 24 h. Then, brine (10 ml) and Aq sat $NaHCO_3$ (10 ml) were added at 0°C and the mixture was stirred for 15 min, filtered through a pad of celite and washed with $CH_2Cl_2$ (20 ml). The solution was decanted and the organic layer was dried and concentrated *in vacuo*. The residue was purified by flash column chromatography ($SiO_2$, EtOAc:MeOH 5:2) to afford **71** (16mg, 65%) as a white solid.

Rf: 0.0.5 (EtOAc:MeOH 5:2).
$^1H$ NMR (300 MHz, $CDCl_3$) d 6.50 (s, 1H), 5.95 (d, $J$=1.5 Hz, 1H), 5.78 (s, 1H), 5.19 (bs, 1H), 4.45 (d, $J$=3.3 Hz, 1H), 4.37 (bs, 1H), 4.11 (brd, $J$=4.8 Hz, 1H), 4.01 (d, $J$=2.1 Hz, 1H), 3.76 (s, 1H), 3.71-3.69 (m, 1H), 3.49-3.35 (m, 1H), 3.24 (d, $J$=13.5 Hz, 1H), 3.15 (d, $J$=9.3 Hz, 1H), 2.95 (dd, $J_1$=8.1 Hz, $J_2$=17.7 Hz, 1H), 2.70 (d, $J$=15.6 Hz, 1H), 2.40 (d, $J$=18.0 Hz, 1H), 2.31 (s, 3H), 2.29 (s, 3H), 2.26 (s, 3H), 1.96 (s, 3H), 1.75-1.66 (m, 1H), 1.52-1.17 (m, 2H), 0.66 (t, $J$=7.2 Hz, 3H).

Fermentation Procedures

Example A

Seed medium YMP3 containing 1% glucose; 0.25% beef extract; 0.5% bacto-peptone; 0.25% NaCl; 0.8% $CaCO_3$ was inoculated with 0.1% of a frozen vegetative stock of the microorganism, strain A2-2 of *Pseudomonas fluorescens*, and incubated on a rotary shaker (250 rpm) at 27°C. After 30 h of incubation, the seed culture was added to a agitated-vessel fermentor with a production medium composed of 2% dextrose; 4% mannitol, 2% dried brewer's yeast (*Vitalevor® Biolux, Belgium*); 1% $(NH_4)_2SO_4$; 0.04% $K_2HPO_4$; 0.8 KCl; 0.001% $FeCl_3$; 0.1% L-Tyr; 0.8% $CO_3Ca$; 0.05% PPG-2000; 0.2% anti-foam silicone (ASSAF-100, RHODIA UK). The sterilisation was carried out at 122°C 30 minutes. The volume inoculated was a 2% (v/v). The temperature was 27°C (0 to 16h) and 24°C from 16h to final process (41 hours). The dissolve oxygen-pressure was upper to 25%. The pH was controlled at 6.0 with diluted sulphuric acid since 28 hours till final process. The overpressure was 0.5 bar. A 1% mannitol or sorbitol was added from 16 h to final process

(for two days running) and 2% for three days fermentation-process.

After 41 or 64 hours, the fermentation broth must be extracted for recovery safracin B or KCN treatment in the clarified broth for recovery safracin B - cyano.

Example B

Obtention of safracin B cyano from the crude extract.

A clarification or filtration from the fermentation broth at pH 6 removes the solids. The clarified broth was adjusted a pH 9.5 with diluted sodium hydroxide and extracted twice with 2:1 (v/v) ethyl acetate, methylene chloride or butyl acetate. The extraction was carried out into an agitated-vessel during 20', the temperature of the mixture was maintained at 8 to 10°C. The two phases were separated by a liquid-liquid centrifuge. The organic phase was dried with sodium sulphate anhydrous or frozen and then filtered for removing ice. This organic phase (ethyl acetate layer) was evaporated until obtention of an oil-crude extract.

Example C

Obtention of safracin B cyano from the clarified broth.

A clarification or filtration from the fermentation broth at pH 6 removes the solids. The clarified broth was adjusted at pH 3.9 with concentrated acetic acid. 0.5 grams per litre of KCN are added to the clarified broth an incubated at 20°C during 1 hour with agitation. Then, the temperature was decreased at 15°C and the pH was adjusted at 9.5 with diluted sodium hydroxide and extracted with 2:1.5 (v/v) ethyl acetate. The extraction was carried out into an agitated-vessel during 20 minutes, the temperature of the mixture was maintained at 8 to 10°C. The two phases were separated by a liquid-liquid centrifuge. The organic phase was dried with sodium sulphate anhydrous. This organic phase (ethyl acetate layer) was evaporated until obtention of an oil-crude extract. This extract was purified by flash column chromatography ($SiO_2$, gradient 20:1 to 10: to 5:1 ethyl acetate:methanol) to afford quantitatively compound 2 as a light yellow solid.

Rf: 0.55 (ethyl acetate:methanol5:1); .$t_R$= 19.9 min [HPLC, Delta Pack C4, 5μm, 300 A, 150x3 mm, λ=215 nm, flow= 0.7 ml/min, temp= 50°C, grad.: $CH_3CN$-aq. NaOAc (10mM) 85% - 70% (20')];

$^1H$ NMR (300 Mhz, $CDCl_3$): δ 6.54 (dd, $J_1$ = 4.4Hz, $J_2$ = 8.4 Hz, 1H),6.44 (s, 1H), 4.12 (d, $J$ = 2.4 Hz, 1H), 4.04 (d, $J$ = 2.4 Hz, 1H), 4.00 (s, 3H), 3.87 (bs, 1H), 3.65 (ddd, $J_1$ = 1.5 Hz, $J_2$ = 8.7 Hz, $J_3$ = 9.9 Hz, 1H), 3.35 (br. D, $J$ = 8.4 Hz, 1H), 3.15-2.96 (m, 4H), 2.92 (q, $J$ = 7.2 Hz, 1H), 2.47 (d, $J$ = 18.3 Hz, 1H), 2.29 (s, 3H), 2.18 (s, 3H) 1.83 (s, 3H), 1.64 (ddd, $J_1$ = 2.7 Hz, $J_2$ = 11.1 Hz, $J_3$ = 14.1 Hz, 1H), 0.79 (d, $J$ = 7.2 Hz, 3H);

$^{13}C$ NMR (75 Mhz, $CDCl_3$): δ 186.0 (q), 175.9 (q), 156.2 (q), 146.8 (q), 142.8 (q), 140.7 (q), 136.6 (q), 130.5 (q), 128.8 (q), 127.0 (q), 120.5 (s), 117.4 (q), 116.5 (q), 60.8 (t), 60.4 (s), 58.7 (t), 56.2 (s), 55.7 (s), 54.8 (s), 54.8 (s), 54.4 (s), 50.0 (s), 41.6 (t), 39.8 (d), 25.2 (d), 24.4 (d), 21.2 (t), 15.5 (t), 8.4 (t).

ESI-MS m/z: Calcd for $C_{29}H_{35}N_5O_6$: 549.6. Found $(M+Na)^+$: 572.3.

Example D

A medium (50 l) composed of dextrose (2%), mannitol (4%), dry brewer's yeast (2%), ammonium sulphate (1%), potassium secondary phosphate (0.04%), potassium chloride (0.8%), iron (III) chloride 6-hydrate (0.001%), L-tyrosine (0.1%), calcium carbonate (0.8%), poly- (propylene glycol) 2000 (0.05%) and antifoam ASSAF 1000 (0.2%) was poured into a jar-fermentor with 75 l total capacity and, after sterilisation, inoculated with seed culture (2%) of A2-2 strain (FERM BP-14) and aerated cultivation under agitation was carried out at 27°C to 24°C for 64 hours (aeration of 75 l per minute and agitation from 350 to 500 rpm). The pH was controlled by automatic feeding of diluted sulphuric acid from 27 hours to final process. A 2% mannitol was added from 16 hours to final process. The cultured medium (45 l) thus obtained was, after removal of cells by centrifugation, adjusted to pH 9.5 with diluted sodium hydroxide, extracted with 25 litres of ethyl acetate twice. The mixture was carried out into an agitated-vessel at 8°C for 20 minutes. The two phases were separated by a liquid-liquid centrifuge. The organic phases were frozen at -20°C and filtered for removing ice and evaporated ice and evaporated until obtention of a 40 g oil-dark-crude extract. After

introduction of the cyanide group and purification, 3.0 grams of safracin B cyano were obtained.

Example E

A medium (50 l) composed of dextrose (2%), mannitol (4%), dry brewer's yeast (2%), ammonium sulphate (1%), potassium secondary phosphate (0.02%, potassium chloride (0.2%), Iron (III) chloride 6-hydrate (0.001%, L-tyrosine (0.1%), calcium carbonate (0.8%, poly- (propylene glycol) 2000 (0.05%) and antifoam ASSAF 1000 (0.2%) was poured into a jar-fermentor with 75 l total capacity and, after sterilisation, inoculated with seed culture (2%) of A2-2 strain (FERM BP-14) and aerated cultivation under agitation was carried out at 27°C to 24°C for 41 hours (aeration of 75 l per minute and agitation from 350 to 500 rpm). The pH was controlled by automatic feeding of diluted sulphuric acid from 28 hours to final process. A 1% mannitol was added from 16 hours to final process. The cultured medium (45 l) thus obtained was, after removal of cells by centrifugation, adjusted to pH 3.9 with 200 ml of conc. acetic acid. 25 grams of potassium cyanide 97% were added and after 1 hour of agitation at 20°C, the pH was adjusted to 9.5 with 1500 ml of a solution 10% sodium hydroxide. Then, extracted with 35 litres of ethyl acetate. The mixture was carried out into an agitated -vessel at 8°C for 20 minutes. The two phases were separated by a liquid-liquid centrifuge. The organic phase was dried by sodium sulphate anhydrous and evaporated until obtention of a 60 g oil-dark-crude extract.

After chromatography, 4.9 grams of safracin B cyano were obtained.

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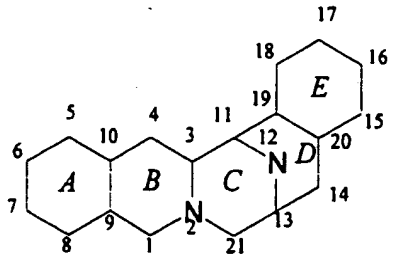
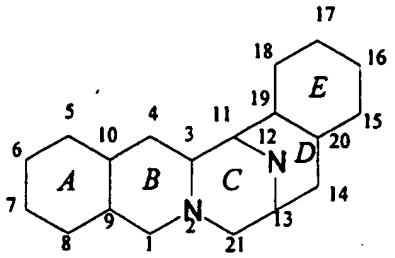
Claims

1. The use in synthesis as starting material of a 21-Nuc compound with a structure of formula (XIV):

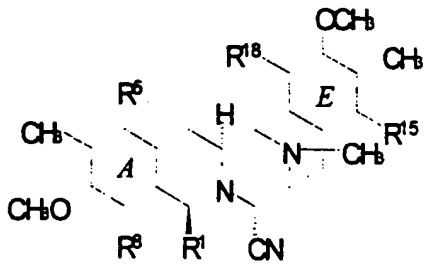


where at least one ring *A* or *E* is quinolic.

2. A method for preparing a compound with a fused ring structure of formula (XIV):



which comprises one or more reactions starting from a 21-cyano compound of formula (XVI):



where:

$R^1$ is an amidomethylene group or an acyloxymethylene group ;

$R^5$ and $R^8$ are independently chosen from -H, -OH or $-OCOCH_2OH$, or $R^5$ and $R^8$ are both keto and the ring *A* is a p-benzoquinone ring;

$R^{14a}$ and $R^{14b}$ are both -H or one is -H and the other is -OH, $-OCH_3$ or $-OCH_2CH_3$, or $R^{14a}$ and $R^{14b}$ together form a keto group; and

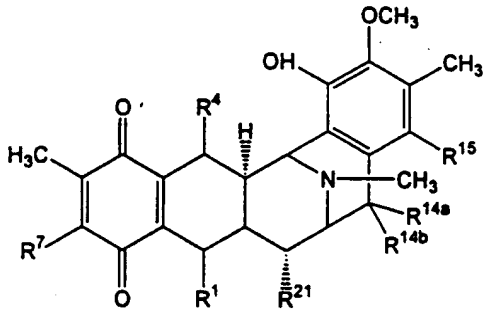
$R^{15}$ and $R^{18}$ are independently chosen from -H or -OH, or $R^5$ and $R^8$ are both keto and the ring

*A* is a p-benzoquinone ring.

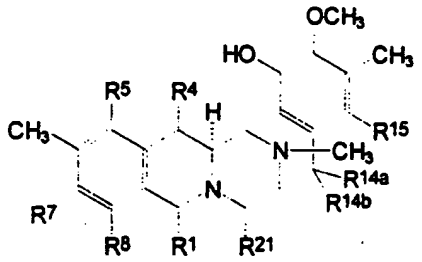
3. A method according to claim 2, where $R^1$ is $-CH_2-NH-CO-CR^{25a}R^{25b}R^{25c}$ where $R^{25a}$ and $R^{25b}$ form a keto group or one is -OH, $-NH_2$ or $-OCOCH_3$ and the other is $-CH_2COCH_3$, -H, -OH or $-OCOCH_3$, provided that when $R^{25a}$ is -OH or $-NH_2$ then $R^{25b}$ is not -OH, and $R^{25c}$ is -H, $-CH_3$ or $-CH_2CH_3$, or $R^1$ is $-CH_2-O-CO-R$, where R is $-C(CH_3)=CH-CH_3$ or $-CH_3$;

4. A method according to claim 2, wherein the 21-cyano compound of formula (XVI) is cyanosafracin B.

5. A method according to any preceding claim, wherein the compound with a fused ring structure of formula (XIV) is a compound of formula (XVIIa):




or formula (XVIIb):



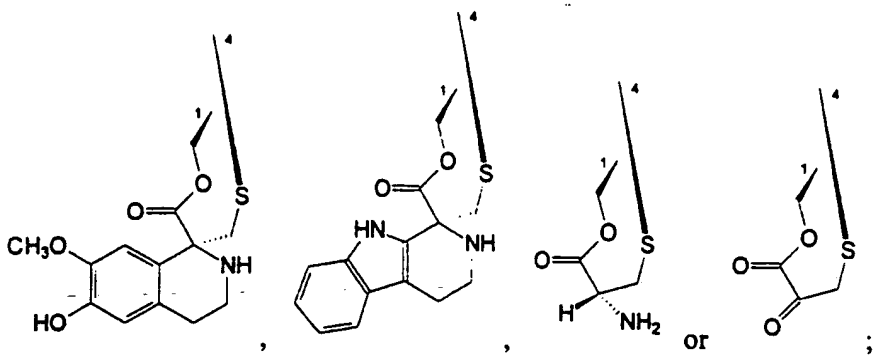

where

$R^1$ is an optionally protected or derivatised aminomethylene group, an optionally protected or derivatised hydroxymethylene group;

$R^4$ is -H;

or

$R^1$ and $R^4$ together form a group of formula (IV), (V) (VI) or (VII):



$R^5$ is -H or -OH;

$R^7$ is $-OCH_3$ and $R^8$ is -OH or $R^7$ and $R^8$ together form a group $-O-CH_2-O-$;

$R^{14a}$ and $R^{14b}$ are both -H or one is -H and the other is -OH, $-OCH_3$ or $-OCH_2CH_3$, or $R^{14a}$ and $R^{14b}$ together form a keto group; and

$R^{15}$ is -H or -OH;

$R^{21}$ is -H, -OH or -CN;

and derivatives including acyl derivatives thereof and including derivatives where the group $-NCH_3-$ at the 12-position is replaced by -NH- or $-NCH_2CH_3-$, and derivatives where the $-NH_2$ group in the compound of formula (VI) is optionally derivatised.

6 A method according to claim 6, wherein $R^5$ is alkanoyloxy of 1 to 5 carbon atoms.

7. A method according to claim 6, wherein R5 is acetyloxy.

8. A method according to claim 5, 6 or 7, wherein $R^{14a}$ and $R^{14b}$ are hydrogen.

9. A method according to any of claims 5 to 8, wherein $R^{15}$ is hydrogen.

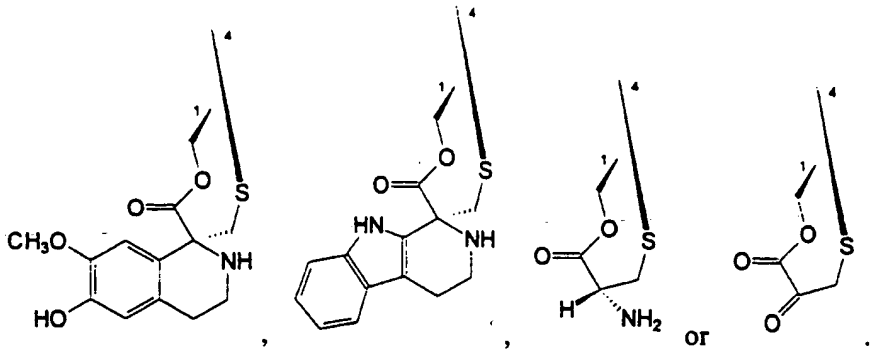
10. A method according to any of claims 5 to 9, wherein $R^{21}$ is -OH or -CN.

11. A method according to any of claims 5 to 10, which is of formula (XVIIb).

12. A method according to claim 11, wherein $R^7$ and $R^8$ together form a group $-O-CH_2-O-$.

13. A method according to any of claims 4 to 11, wherein $R^1$ and $R^4$ together form a group

of formula (IV), (V) (VI) or (VII):



14 A method according to any of claims 5 to 12, wherein $R^1$ is an optionally protected or derivatised aminomethylene group, an optionally protected or derivatised hydroxymethylene group; and $R^4$ is -H.

15 A method according to claim 14, wherein $R^1$ is a group $-CH_2NH_2$ or $-CH_2$-NH-aa, where aa is an acyl amino acid group.

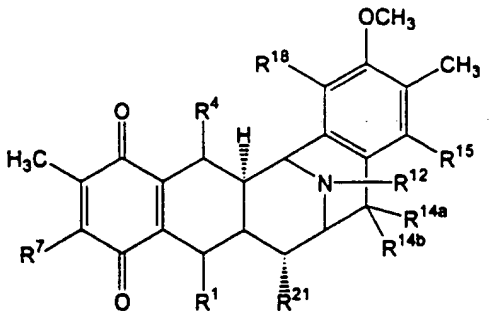
16. A method according to claim 15, wherein in the form of an N-acyl derivative of the group $-CH_2NH_2$ or $-CH_2$-NH-aa.

17. A method according to claim 16, wherein $R^1$ is a N-acyl derivative where the acyl group is of formula $-CO-R^a$, where $R^a$ is alkyl, alkoxy, alkylene, arylalkyl, arylalkylene, amino acid acyl, or heterocyclyl; each optionally substituted with halo, cyano, nitro, carboxyalkyl, alkoxy, aryl, aryloxy, heterocyclyl, heterocyclyloxy, alkyl, amino or substituted amino; or $R^a$ is aa.

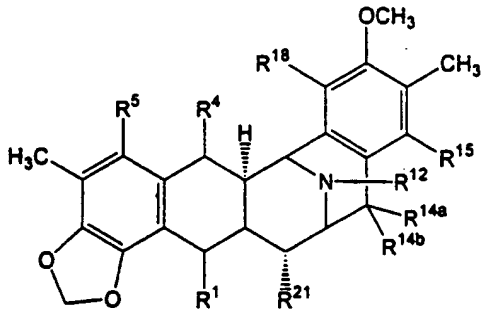
18. A method according to claim 15, 16 or 17, wherein one or more aa groups is present and is alanyl, arginyl, aspartyl, asparagyl, cystyl, glutamyl, glutaminyl, glycyl, histidyl, hydroxyprolyl., isoleucyl, leucyl, lysyl, methionyl, phenylalanyl, prolyl, seryl, threonyl, thyronyl, tryptophyl, tyrosyl, valyl, or another amino acid acyl group.

19. A method according to any of claims 5 to 18, wherein one or more substituent groups is protected by a protecting group.

20. A method according to any preceding claim, wherein the product is of formula (XXIIa):



or of formula (XXIIb):

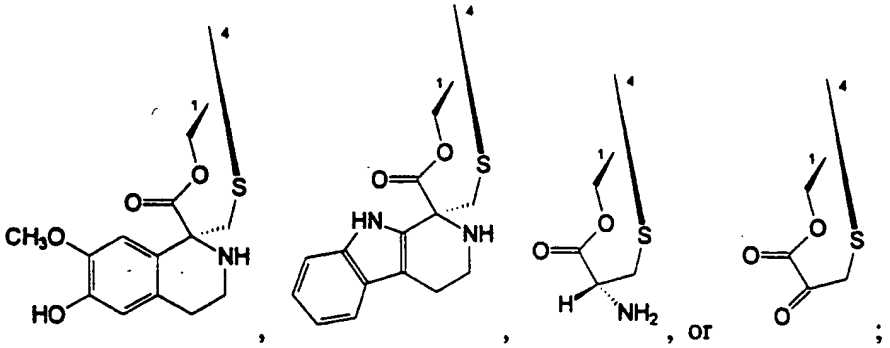


where:

$R^1$ is $-CH_2NH_2$ or $-CH_2OH$, or a protected or derivatised version of such a group and $R^4$ is -H;

or

$R^{1a}$ and $R^4$ together form a group of formula (IV), (VI) or (VII):



;

$R^5$ is -OH or a protected or derivatised version of such a group;

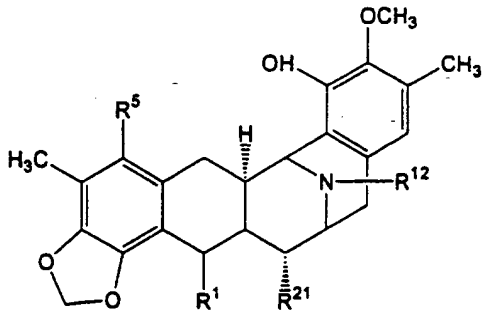
$R^{14a}$ and $R^{14b}$ are both -H or one is -H and the other is -OH or a protected or derivatised version of such a group, $-OCH_3$ or $-OCH_2CH_3$, or $R^{14a}$ and $R^{14b}$ together form a keto group;

$R^{12}$ is -NH-, $-NCH_3-$ or $-NCH_2CH_3-$;

$R^{15}$ is -OH or a protected or derivatised version of such a group; and

$R^{18}$ is -OH or a protected or derivatised version of such a group.

21. A method according to any preceding claim, where the product is of formula (XXIII):




where $R^1$ is as previously defined for formula (XVIIb) and is preferably a derivatised aminomethylene group of moderate bulk;

$R^5$ is as previously defined for formula (XVIIb) and is preferably a derivatised hydroxy group of low bulk;

$R^{12}$ is as previously defined and is preferably $-NCH_3-$ and

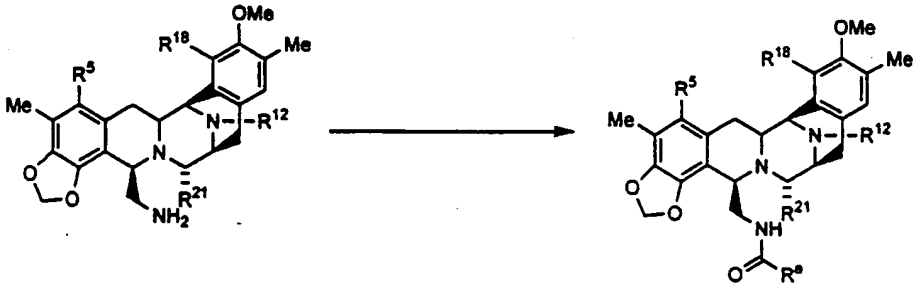
$R^{21}$ is a hydroxy or cyano group.

22. A method according to claim 21, where $R^1$ is a hydrophobic group.

23 A method according to claim 22, where $R^1$ is a group $-CH_2-NH_2-CO-R^a$, where $R^a$ has a linear chain length of less than 20 atoms, more preferably less than 15 or 10 atoms.

24. A method according to claim 20,21 or 22, wherein $R^5$ is an acetyl group.

25. A method according to any preceding claim, which includes the step:




where $R^5$ for the end product is as defined for the compound (XXXII) and may be different in

the starting material and converted thereto as part of the process,

$R^{18}$ is a hydroxy group in the end product but may be a protected hydroxy group in the starting material and converted thereto as part of the process,

$R^{12}$ for the end product may be the same as in the starting material or may be converted thereto as part of the process,

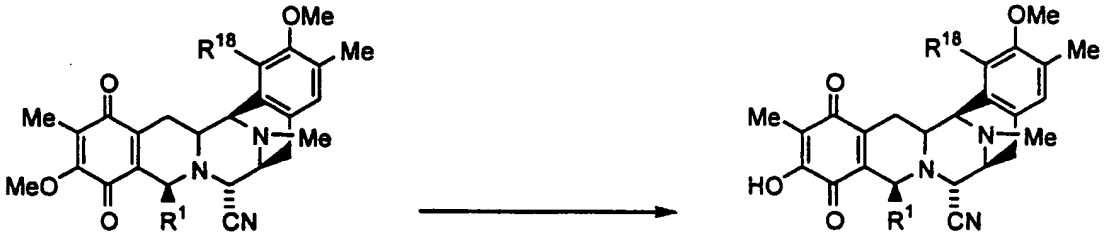
$R^{21}$ for the end product is as defined and if a hydroxy group may be formed from a cyano group as part of the process,

$R^a$ is as defined, and may be further acylated as part of the process to give an end product with an acylated $R^a$ group.

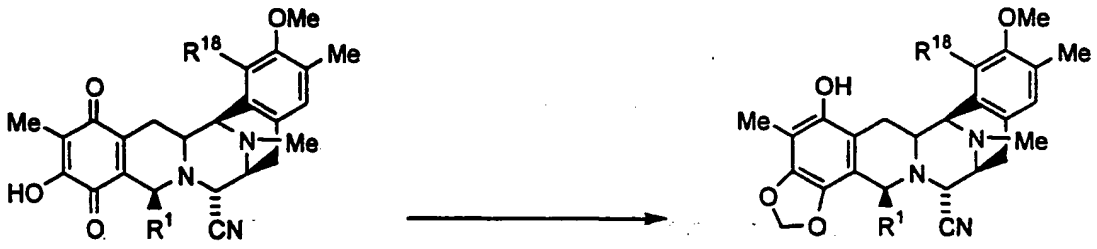
26. A method according to any preceding claim, wherein aa is alanyl.

27. A method according to claim 26, wherein the alanyl group is present in the starting material and is protected with a Boc group.

28. A method according to any preceding claim, which includes the reaction:

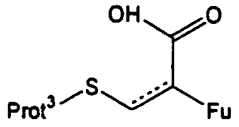



29. A method according to any preceding claim, which includes the reaction:



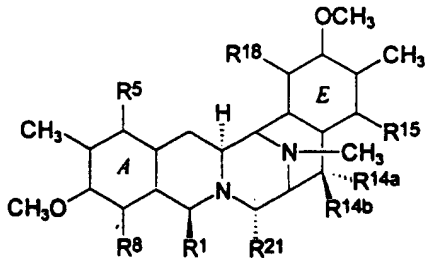

30. A method according to any preceding claim, which includes the reaction where a group $R^1$ is aminomethylene is converted to a hydroxymethylene group.

31. A method according to any preceding claim, wherein a compound with a group $R^1$ which is hydroxymethylene is reacted with a reagent of the formula (XIX)



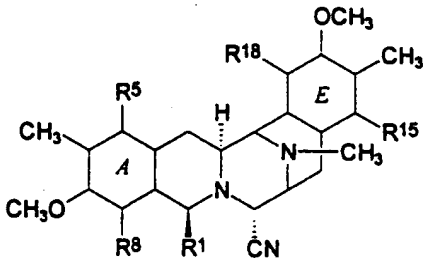
where Fu indicates a protected functional group, $Prot^3$ is a protecting group, and the dotted line shows an optional double bond.

32. A method for preparing a 21-cyano compound of formula (XVI), as defined in claim 1, which comprises reacting a compound of formula (XV):



where $R^1$, $R^5$, $R^8$, $R^{14a}$, $R^{14b}$, $R^{15}$ and $R^{18}$ are as defined and $R^{21}$ is a hydroxy group, with a source of cyanide ion, to give the desired 21-cyano compound.

33. A 21-cyano compound of formula (XVI):



where:

$R^1$ is an amidomethylene group or an acyloxymethylene group ;

$R^5$ and $R^8$ are independently chosen from -H, -OH or $-OCOCH_2OH$, or $R^5$ and $R^8$ are both keto and the ring *A* is a p-benzoquinone ring;

$R^{14a}$ and $R^{14b}$ are both -H or one is -H and the other is -OH, $-OCH_3$ or $-OCH_2CH_3$, or $R^{14a}$ and $R^{14b}$ together form a keto group; and

$R^{15}$ and $R^{18}$ are independently chosen from -H or -OH, or $R^5$ and $R^8$ are both keto and the ring

*A* is a p-benzoquinone ring,

with the exception of safracin B.

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International Bureau

(43) International Publication Date
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PCT

(10) International Publication Number
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| 9918178.6 | 2 August 1999 (02.08.1999) | GB |
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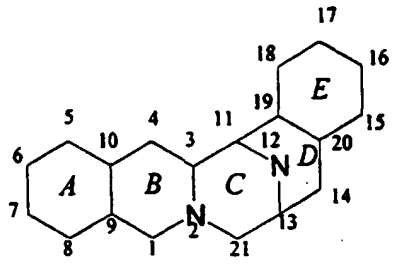
(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **CUEVAS, Carmen** [ES/ES]; Pharma Mar, S.A., Calle de la Calera, 3, Poligono Industrial de Tres Cantos, Tres Cantos, E-28760 Madrid (ES). **PEREZ, Marta** [ES/ES]; Pharma Mar, S.A., Calle de la Calera, 3, Poligono Industrial de Tres Cantos, Tres Cantos, E-28760 Madrid (ES). **FRANCESCH, Andres** [ES/ES]; Pharma Mar, S.A., Calle de la Calera, 3, Poligono Industrial de Tres Cantos, Tres Cantos, E-28760 Madrid (ES). **FERNANDEZ, Carolina** [ES/ES]; Pharma Mar, S.A., Calle de la Calera, 3, Poligono Industrial de Tres Cantos, Tres Cantos, E-28760 Madrid (ES). **CHICHARRO, Jose Luis** [ES/ES]; Pharma Mar, S.A., Calle de la Calera, 3, Poligono Industrial de Tres Cantos, Tres Cantos, E-28760 Madrid (ES). **GALLEGO, Pilar** [ES/ES]; Pharma Mar, S.A., Calle de la Calera, 3, Poligono Industrial de Tres Cantos, Tres Cantos, E-28760 Madrid (ES). **ZARZUELO, Maria** [ES/ES]; Pharma Mar, S.A., Calle de la Calera, 3, Poligono Industrial de Tres Cantos, Tres Cantos, E-28760 Madrid (ES). **DE LA CALLE, Fernando** [ES/ES]; Pharma Mar, S.A., Calle de la Calera, 3, Poligono Industrial de Tres Cantos, Tres Cantos, E-28760 Madrid (ES). **MANZANARES, Ignacio** [ES/ES]; Pharma Mar, S.A., Calle de la Calera, 3, Poligono Industrial de Tres Cantos, Tres Cantos, E-28760 Madrid (ES).

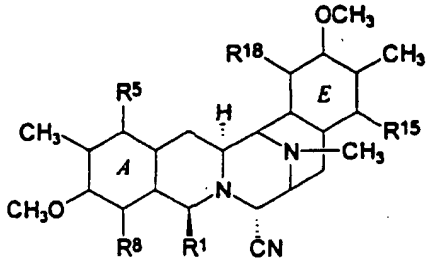
(74) Agent: **RUFFLES, Graham, Keith**; Marks & Clerk, 57-60 Lincoln's Inn Fields, London WC2A 3LS (GB).

*[Continued on next page]*

(54) Title: HEMISYNTHETIC METHOD AND INTERMEDIATES THEREOF




(XIV)




(XVI)

(57) Abstract: Methods are provided for preparing a compound with a fused ring structure of formula (XIV) which comprises one or more reactions starting from a 21-cyano compound of formula (XVI) where typically: $R^1$ is an amidomethylene group or an acyloxymethylene group; $R^5$ and $R^8$ are independently chosen from -H, -OH or $-OCOCH_2OH$, or $R^5$ and $R^8$ are both keto and the ring *A* is a p-benzoquinone ring; $R^{14a}$ and $R^{14b}$ are both -H ozone is -H and the other is -OH, $-OCH_3$ or $-OCH_2CH_3$, or $R^{14a}$ and $R^{14b}$ together form a keto group; and $R^{15}$ and $R^{18}$ are independently chosen from -H or -OH, or $R^5$ and $R^8$ are both keto and the ring *A* is a p-benzoquinone ring. In modified starting materials, the 21-cyano group can be replaced by other groups introduced using nucleophilic reagents.

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(81) **Designated States** *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) **Designated States** *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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# INTERNATIONAL SEARCH REPORT

Interm ıal Application No
PCT/GB 00/01852

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D515/22 C07D491/22 C07D471/18 A61K35/00
//(C07D515/22,317:00,291:00,241:00,221:00,221:00),(C07D491/22,
317:00,241:00,221:00,221:00),(C07D471/18,241:00,221:00,221:00)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 5 December 2000 | 12/12/2000 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Goss, I |

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